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Induction of Reproductive Diapause in *Habrobracon hebetor* (Hymenoptera: Braconidae) When Reared at Different Photoperiods at Low Temperatures

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ABSTRACT Development of the parasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) at low temperatures was determined to identify rearing conditions that might result in adults that were in reproductive diapause. Diapausing adults would be expected to survive cold storage longer than nondiapausing adults for use in biological control programs. Only a few eggs were found in the ovaries when *H. hebetor* females were reared during the immature stages at 17.5 and 20°C with a 16-h photoperiod, and the ovaries were poorly developed and contained no eggs when females were reared with a 10-h photoperiod in these low temperatures. Rearing *H. hebetor* at 17.5 and 20°C did not result in diapause of immature stages, but did appear to result in possible adult reproductive diapause when the immature stages were reared with a 10-h photoperiod. Females reared during the immature stages at 17.5°C with a10-h photoperiod lived longer and took longer to lay their first eggs and to lay 50% of their eggs than those females reared at 17.5°C with a16-h photoperiod. Females reared during the immature stages at 20°C with a10-h photoperiod took longer to lay their first eggs and to lay 50% of their eggs, and they had a lower respiration rate, than those females reared at 20°C with a16-h photoperiod. Females that were reared in conditions that appeared to induce reproductive diapause resumed oviposition and their respiration rate increased soon after being transferred to a higher temperature (27.5°C). Thus, females reared at a 10-h photoperiod at 17.5 and 20°C appear to enter reproductive diapause.

KEY WORDS biological control, ectoparasitoid, *Habrobracon hebetor*, photoperiod, reproductive diapause

Habrobracon hebetor (Say) (*Bracon hebetor*) (Hymenoptera: Braconidae) is a cosmopolitan ectoparasitoid that is considered a potential biological control agent of various lepidopteran pests (Press et al. 1982, Balevski 1984, Huang 1986, Keever et al. 1986, Brower and Press 1990, Amir-Maafi and Chi 2006). It has been used for suppressing moth populations in stored products (Press et al. 1982, Balevski 1984, Huang 1986, Brower and Press 1990, Cline and Press 1990, Garba and Gaoh 2008) and in field crops (Gerling 1971, Cheng 1991, Uwais et al. 2006, Imam et al. 2007).

One obstacle to the use of insect natural enemies for biological control can be obtaining sufficient numbers at the time that they are required for release (Coudron et al. 2007). Cold storage of natural enemies is a possible strategy for overcoming this problem, and parasitoids from the family Braconidae usually are stored as adults (Leopold 1998).

Diapause is one of the main strategies used by insects to survive unfavorable environmental conditions, and it is generally accepted that diapausing insects can be stored longer at low temperatures than nondiapausing insects (Havelka 1980) without a reduction in performance (Canard 1971, Gilkeson 1990, Tauber et al. 1993). Temperature and photoperiod are the two main factors that regulate induction of diapause in insects (Kobayashi and Numata 1995, Wei et al. 2001, Musolin et al. 2004, Wang et al. 2004). The effects of temperature (Garcia et al. 2002, Margaritopoulos and Tzanakakis 2006, Reznik et al. 2008), photoperiod (Ma and Chen 2006), and their interaction (Milonas and Savopoulou-Soultani 2000, Li et al. 2008) on diapause induction have been studied in some species of Hymenoptera. Many parasitoids overwinter as adults (Rasnitsyn 1964, Simmons and Nelson 1975, David 1988, Johnson et al. 2000), but little work has

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Table 1. Stage-specific survival (mean % ± SE) of *H. hebetor* reared at different photoperiods and temperatures

Stage	Photoperiod (h)			*F*	df	*P*
	10	13	16			
17.5°C						
Egg	80.3 ± 4.1a		82.9 ± 3.0a	0.3	1,4	0.635
Larva	85.1 ± 4.2a		83.1 ± 6.1a	0.08	1,4	0.792
Pupa	100 ± 0a		100 ± 0a			
Overall	68.0 ± 1.8a		68.5 ± 2.5a	0.02	1,4	0.891
20°C						
Egg	82.5 ± 4.2a	85.5 ± 3.3a	83.8 ± 4.4a	0.1	2,6	0.870
Larva	86.9 ± 4.9a	89.8 ± 0.8a	86.1 ± 2.4a	0.4	2,6	0.694
Pupa	99.1 ± 0.9a	99.2 ± 0.8a	100 ± 0a	0.5	2,6	0.627
Overall	70.6 ± 1.0a	76.1 ± 2.6a	72.0 ± 3.0a	1.5	2,6	0.300

Means within a row followed by the same letter are not significantly different at $P < 0.05$ (ANOVA with Tukey's-b).

been done on induction of diapause in adult parasitoids in general (Numata 1993, Tatsumi and Takada 2005) and in *H. hebetor* in particular (Adashkevich and Saidova 1985). Our objectives were to determine rearing conditions for *H. hebetor* that would induce diapause, and to evaluate the performance of *H. hebetor* adult females reared during the immature stages at different photoperiods in low temperatures that were expected to induce diapause. In particular, we wanted to determine rearing temperatures that were high enough for fairly rapid population development while still inducing diapause, so that the method would be useful in insectaries.

Materials and Methods

Insect Rearing for Host and Parasitoid. The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), was maintained in glass jars (500 ml) on an artificial diet consisting of cracked wheat (1,000 g); wheat shorts (1,000 g); wheat germ (100 g); brewer's yeast (80 g); glycerine (240 ml); honey (240 ml); and 120 ml of water (McGaughey and Beeman 1988). Pupae were collected from the stock culture by placing rolls of corrugated cardboard (2 cm high by 5.7 cm in diameter) in the rearing jars for larvae to crawl into to pupate. A cardboard roll containing pupae then was placed in a new glass jar for adults to emerge and lay eggs. We then transferred ≈50 mg of eggs (≈2,000 eggs) to a 500-ml glass jar filled to about one-third with artificial diet. Environmental conditions for the culture were 30.0 ± 0.5°C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h. Last instars were used for experiments.

A field population of *H. hebetor* used in the experiments was collected in Parlier, CA, in October 2009, and experiments were conducted during the next year. Pairs of adult parasitoids were introduced into 55.5-ml plastic vials (3.2 cm in diameter by 8.3 cm high) containing 30 *P. interpunctella* last instars, and the vials were capped with lids that had a 12-mm-diameter hole covered with fine screening. Environmental conditions for rearing *H. hebetor* were 27.5 ± 0.5°C, 65 ± 5% RH, with a photoperiod of 18:6 (L:D) h. To obtain *H. hebetor* eggs for experiments, 30 pairs of <48-h-old males and females were allowed to mate for 2 d in 55.5-ml plastic vials containing 100 last-instar *P. interpunctella*, and then individual females were removed from the vials and allowed to oviposit for 24 h on nine last-instar *P. interpunctella* in 55.5-ml vials. We determined in preliminary tests that providing nine larvae per female for 24 h usually resulted in no more than three eggs being laid on a larva, and Strand and Godfray (1989) reported that there were sufficient resources in a single larva to support development of four *H. hebetor*. The number of eggs on a larva was counted, and larvae with no more than three eggs were selected for experiments to ensure that there were adequate nutritional resources available for development.

Rearing Conditions for Diapause Induction. Different rearing conditions (20°C, 65% RH, and 16-, 13-, or 10-h photoperiod; or 17.5°C, 65% RH, and 16- or 10-h photoperiod) were maintained in Percival I-36VL incubators (Percival Scientific, Inc., Perry, IA). Experiments at the two temperatures were conducted sequentially. We chose these temperatures

Table 2. Developmental time (mean days ± SE) of immature *H. hebetor* reared at different photoperiods and temperatures

	Photoperiod (h)			*F*	df	*P*
	10	13	16			
17.5°C						
Male	33.1 ± 0.3a		32.3 ± 0.3a	3.4	1,85	0.069
Female	33.9 ± 0.3a		32.6 ± 0.2b	13.8	1,114	< 0.001
Male + female	33.5 ± 0.2a		32.5 ± 0.1b	14.3	1,201	< 0.001
20°C						
Male	26.1 ± 0.2a	24.8 ± 0.2b	23.8 ± 0.2c	33.1	2,202	< 0.001
Female	27.4 ± 0.2a	25.4 ± 0.2b	24.8 ± 0.3b	25.8	2,120	< 0.001
Male + female	26.6 ± 0.2a	25.0 ± 0.1b	24.2 ± 0.2c	55.7	2,325	< 0.001

Means within a row followed by the same letter are not significantly different at $P < 0.05$ (ANOVA with Tukey's-b).

Table 3. Longevity (mean days ± SE) of *H. hebetor* adults reared at different temperatures and photoperiods and then kept at rearing conditions or moved to 27.5°C and a 16-h photoperiod

Rearing conditions	Oviposition conditions		*F*	df	*P*
	Same as rearing conditions	27.5°C, 16:8 (L:D) h			
17.5°C					
10:14 (L:D) h	67.7 ± 4.8Aa	21.0 ± 1.5Bb	86.9	1,38	<0.001
16:8 (L:D) h	38.3 ± 3.4Ba	27.5 ± 2.5Ab	6.6	1,38	0.015
F	25.0	5.0			
df	1,38	1,38			
P	<0.001	0.032			
20°C					
10:14 (L:D) h	80.5 ± 4.6Aa	34.3 ± 2.7Ab	76.4	1,38	<0.001
13:11 (L:D) h	64.8 ± 6.4Aa	32.4 ± 3.0Ab	20.4	1,37	<0.001
16:8 (L:D) h	64.7 ± 6.6Aa	31.3 ± 2.7Ab	24.3	1,35	<0.001
F	2.5	0.7			
df	2,56	2,54			
P	0.090	0.520			

Means within a column and temperature followed by the same uppercase letter or those within a row followed by the same lowercase letter are not significantly different at $P < 0.05$ (ANOVA with Tukey's-b).

because we hypothesized that diapause would occur at these temperatures at shorter photoperiods (Brodeur and McNeil 1989), whereas we expected that developmental time still would be fast enough to enable rearing in an insectary to produce parasitoids in reasonable numbers for cold storage. Diapause induction also would be expected to occur at temperatures lower than these, but developmental time would be slow. Petri dishes containing *P. interpunctella* larvae with ≈50 *H. hebetor* eggs were placed at each condition, and there were three replicates. Developmental stage was determined every 3.5 d (twice a week) to minimize disturbance to the parasitoids and to the temperature and light conditions. After emergence, females were moved individually into a 55.5-ml vial, and they were left at their treatment conditions for 3.5 d before physiological parameters were determined (as described in the next section).

Physiological Parameters Determined. After the 3.5–7.0-d postemergence period, individual females from each condition were introduced into 55.5-ml vials containing nine last-instar *P. interpunctella* with individual 0- to 1-wk-old males from a different petri dish. Twenty of these pairs were kept at their rearing conditions, and 20 pairs from each condition were moved to a chamber set at 27.5°C, 65% RH, and a photoperiod of 16:8 (L:D) h. The adults were moved to a new vial set up identically to the previous one every 3.5 d until the parental female died. Dead males were not replaced. The *P. interpunctella* larvae in the old vials were checked to determine the number of eggs laid.

Other females were dissected using a method adapted from Howard et al. (2003) after the 3.5–7.0-d postemergence period to determine development of the ovaries. Adult females were chilled at −20°C for 3 min, and then they were dissected in a black depression plate. Forceps were used to hold the wasp firmly by the thorax under the surface of a saline solution, and then ophthalmic scissors were used to cut along the side of the abdomen. The reproductive system, as shown in Genieys (1925), was observed easily after removing the abdominal sclerites and fat body so that the developmental stage of the ovaries could be determined.

Table 4. Duration of the preoviposition period (mean days ± SE) of *H. hebetor* reared at different temperatures and photoperiods and then kept at rearing conditions or moved to 27.5°C and a 16-h photoperiod

Rearing conditions	Oviposition conditions		*F*	df	*P*
	Same as rearing conditions	27.5°C, 16:8 (L:D) h			
17.5°C					
10:14 (L:D) h	24.7 ± 2.1Aa	0.4 ± 0.4Ab	129.0	1,37	<0.001
16:8 (L:D) h	0Ba	0Aa			
F	144.2	1.1			
df	1,38	1,37			
P	<0.001	0.311			
20°C					
10:14 (L:D) h	15.4 ± 1.5Aa	0.2 ± 0.2Ab	109.4	1,38	<0.001
13:11 (L:D) h	1.5 ± 0.5Ba	0Ab	12.9	1,37	0.001
16:8 (L:D) h	0.2 ± 0.2Ba	0Aa	0.9	1,35	0.337
F	87.0	0.9			
df	2,56	2,54			
P	<0.001	0.404			

Means within a column and temperature followed by the same uppercase letter or those within a row followed by the same lowercase letter are not significantly different at $P < 0.05$ (ANOVA with Tukey's-b).

Table 5. Number (mean ± SE) of eggs laid by *H. hebetor* reared at different temperatures and photoperiods and then kept at rearing conditions or moved to 27.5°C and a 16-h photoperiod

Rearing conditions	Oviposition conditions		*F*	df	*P*
	Same as rearing conditions	27.5°C, 16:8 (L:D) h			
17.5°C					
10:14 (L:D) h	57.1 ± 11.3Aa	153.3 ± 20.1Ab	17.4	1,38	<0.001
16:8 (L:D) h	118.6 ± 18.4Ba	238.4 ± 26.8Bb	13.6	1,38	0.001
F	8.1	6.4			
df	1,38	1,38			
P	0.007	0.015			
20°C					
10:14 (L:D) h	256.0 ± 24.4Aa	300.4 ± 34.1Aa	1.1	1,38	0.296
13:11 (L:D) h	243.2 ± 32.0Aa	279.5 ± 30.5Aa	0.7	1,37	0.417
16:8 (L:D) h	309.0 ± 37.4Aa	283.1 ± 17.4Aa	1.5	1,35	0.230
F	1.2	0.6			
df	2,56	2,54			
P	0.306	0.547			

Means within a column and temperature followed by the same uppercase letter or those within a row followed by the same lowercase letter are not significantly different at $P < 0.05$ (ANOVA with Tukey's-b).

Respiration rate of female wasps, each weighing 1 ± 0.1 mg and reared at 20°C, 65% RH, and 16- or 10-h photoperiod, was determined after the 3.5–7.0-d postemergence period. Each wasp was placed in a small vial capped with a fine mesh screen, which then was placed in a sealed bottle with another vial filled with saturated sodium chloride solution to control the relative humidity at 75% (Greenspan 1977). Respiration rate was determined at 20 and 27.5°C (± 0.5°C) by placing the bottles in a temperature-adjustable water bath. Each bottle was connected to an individual channel in a closed circuit respirometer system (Micro Oxymax, Columbus Instruments, Columbus, OH) interfaced to a desktop computer. One bottle contained a vial filled with saturated sodium chloride solution to serve as a control. Oxygen consumption and carbon dioxide production were determined at 2-h intervals for 72 h.

Statistical Analysis. Stage-specific survival rate, developmental time, duration of the preoviposition period, longevity, oviposition, and respiration rate were compared within a temperature by using one-way analysis of variance (ANOVA) and Tukey's-b Test ($P = 0.05$) (SPSS Inc. 2007).

Results

Stage-specific survival rate during immature development did not differ among photoperiods within a temperature (Table 1). Most mortality occurred during the egg and larval stages, whereas almost all pupae survived to the adult stage.

Developmental time of immature *H. hebetor* decreased as photoperiod increased when parasitoids were reared at 20°C (Table 2). The difference in developmental times at the two photoperiods at 17.5°C was small and only significant for females and for the two sexes combined (Table 2).

Parasitoids always lived longer as adults when left in their rearing conditions (low temperature) than when they were moved to 27.5°C (Table 3). There were no differences in adult longevity when parasitoids were

Table 6. Time to 50% oviposition (mean days ± SE) of *H. hebetor* reared at different temperatures and photoperiods and then kept at rearing conditions or moved to 27.5°C and a 16-h photoperiod

Rearing conditions	Oviposition conditions		*F*	df	*P*
	Same as rearing conditions	27.5°C, 16:8 (L:D) h			
17.5°C					
10:14 (L:D) h	38.9 ± 3.3Aa	11.7 ± 0.8Ab	64.0	1,38	<0.001
16:8 (L:D) h	15.9 ± 2.1Ba	12.1 ± 0.9Aa	2.8	1,38	0.102
F	34.1	0.09			
df	1,38	1,38			
P	<0.001	0.764			
20°C					
10:14 (L:D) h	43.9 ± 2.4Aa	16.8 ± 0.9Ab	110.7	1,38	<0.001
13:11 (L:D) h	27.8 ± 2.2Ba	15.7 ± 1.3Ab	23.2	1,37	<0.001
16:8 (L:D) h	23.8 ± 2.2Ba	13.8 ± 1.2Ab	14.9	1,35	<0.001
F	22.0	1.8			
df	2,56	2,54			
P	<0.001	0.174			

Means within a column and temperature followed by the same uppercase letter or those within a row followed by the same lowercase letter are not significantly different at $P < 0.05$ (ANOVA with Tukey's-b).

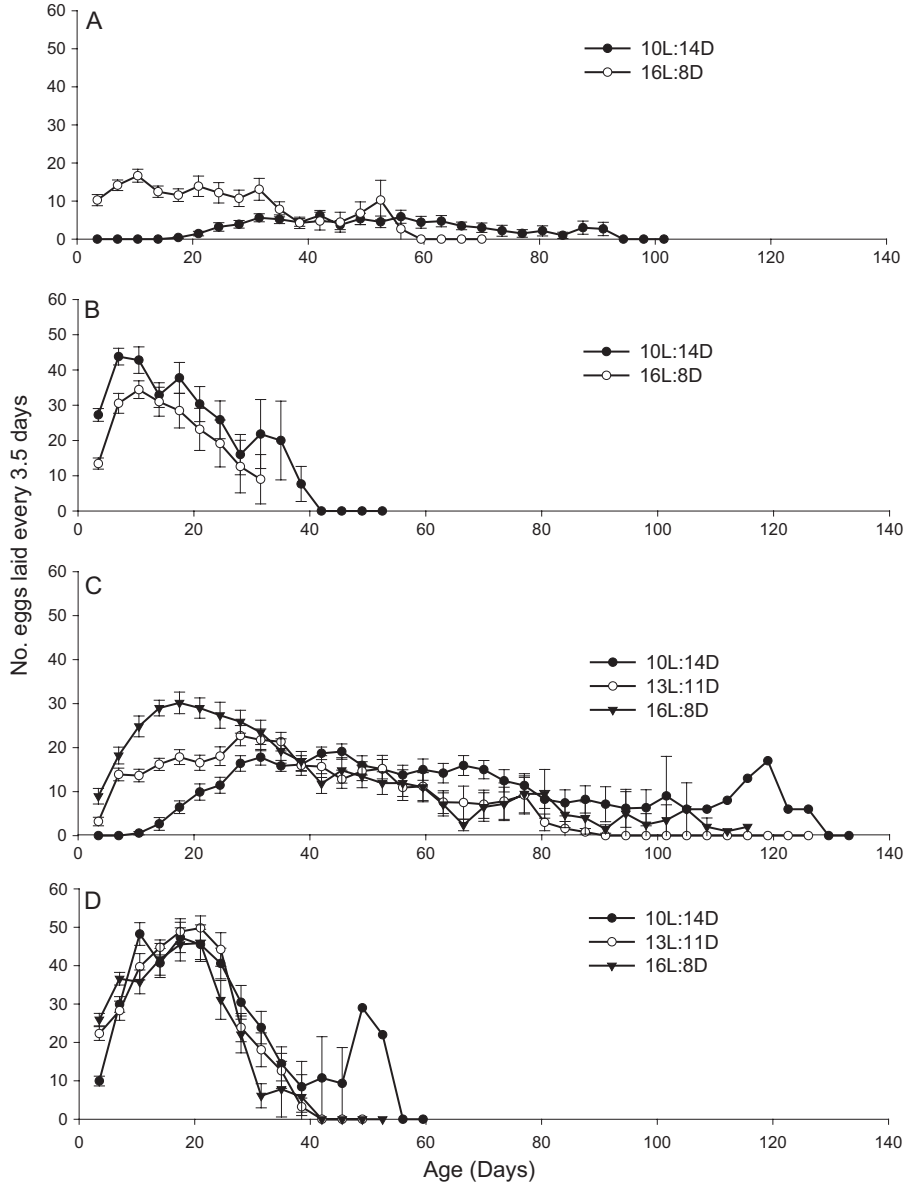

Fig. 1. Oviposition of *H. hebetor* adults reared at different photoperiods and temperatures. (A) Rearing and oviposition at 17.5°C at two different photoperiods. (B) Rearing at two different photoperiods at 17.5°C and then moved to 27.5°C and a 16-h photoperiod for oviposition. (C) Rearing and oviposition at 20°C at three different photoperiods. (D) Rearing at three different photoperiods at 20°C and then moved to 27.5°C and a 16-h photoperiod for oviposition.

reared at the different photoperiods at 20°C, either when they were left at those rearing conditions or when they were moved to 27.5°C. However, parasitoids reared with a 10-h photoperiod lived 29.4 d longer than those reared with a 16-h photoperiod when both sets were left in their rearing conditions at 17.5°C. Conversely, parasitoids reared at 17.5°C with a 16-h photoperiod lived longer than those reared at 17.5°C with a 10-h photoperiod when moved as adults to 27.5°C, although this difference was only 6.5 d.

Preoviposition period was short except when parasitoids were reared at a 10-h photoperiod and left to lay eggs at their rearing conditions (Table 4). In other treatments, almost all of the females had laid eggs by the first observation time. There were no differences in number of eggs laid among any of the photoperiods when females were reared at 20°C (Table 5), whether they were left at their rearing conditions or moved as adults to 27.5°C. Females reared at 17.5°C laid more eggs when moved as adults to 27.5°C, and females reared at 17.5°C at the 16-h photoperiod laid more eggs than those reared at the 10-h photoperiod.

Females that were moved as adults to 27.5°C laid 50% of their eggs more quickly than those left at their

Table 7. Respiration (mean CO_2 accumulation [ul] ± SE) after 24–72 h of female *H. hebetor* at 20 and 27.5°C when reared at short and long photoperiods at 20°C

Temperature (°C)	Time (h)	Rearing photoperiod (h)		*F*	df	*P*
		10	16			
20	24	9.0 ± 0.4a	11.7 ± 0.3b	32.9	1,22	<0.001
	48	16.7 ± 0.7a	21.9 ± 0.4b	37.0	1,22	<0.001
	72	24.3 ± 1.1a	32.3 ± 0.8b	35.1	1,22	<0.001
27.5	24	29.0 ± 2.0a	31.7 ± 2.1a	0.8	1,22	0.374
	48	54.7 ± 3.2a	56.7 ± 4.0a	0.1	1,22	0.704
	72	76.1 ± 4.7a	77.2 ± 5.6a	0.02	1,22	0.888

Means within a row followed by the same letter are not significantly different at $P < 0.05$ (ANOVA with Tukey's-b).

rearing conditions, except for females reared at 17.5°C with a 16-h photoperiod where there was no difference between those left at their rearing conditions or moved to 27.5°C (Table 6; Fig. 1). There were no differences in time to 50% egg laying among females reared at the different photoperiods when they were moved to 27.5°C; however, females reared at the 10-h photoperiod and left at rearing conditions took longer to lay 50% of their eggs than females reared at a longer photoperiod.

Females reared at 20°C with a 16-h photoperiod had a higher respiration rate than those females reared at a 10-h photoperiod when tested at 20°C; however, the respiration rate didn't differ between photoperiods when the females were tested at 27.5°C (Table 7). The respiration rate was about three times higher when females were moved to 27.5°C than when left at the conditions at which they were reared.

As expected, we observed that each female had two ovaries, one on each side of the body, and each ovary had a pair of ovarioles (Fig. 2). When reared during the immature stages at 27.5°C, the ovary was well developed, full of eggs, and occupied most of the abdomen (Fig. 2A). When females were reared at 17.5 or 20°C and a 16-h photoperiod, a smaller number of eggs were found in the ovaries than were found when the females were reared at 27.5°C (Figs. 2B and 2E). When females were reared at 17.5 or 20°C at a 10-h photoperiod, the ovaries became small convoluted tubes, no eggs were present, and the fat body filled the abdomen (Figs. 2C and 2D).

Discussion

The most common criteria to indicate induction of reproductive diapause in female insects is the lack of mature eggs or vitellogenic oocytes in ovarioles and the lack of oviposition (Andrewartha 1952, Hodek 1965, Nakamura and Numata 1995, Musolin et al. 2004). Respiration rate also declines when the insect enters diapause, regardless of which stage enters diapause (Fielding 2008). A common criterion for termination of reproductive diapause in females is the resumption of oviposition (Beck 1980). Generally, the ovaries of females in reproductive diapause will develop rapidly when the females are moved to a higher temperature, as was reported for the nymphalid *Danaus plexippus* (L.) from overwintering clusters in Australia (James 1982). Similarly, adult diapause in *Drosophila melanogaster* Meigen was terminated rapidly after being transferred to higher temperatures (15 or 18°C) or to long-day conditions after adult diapause was induced by short-day conditions at 12°C (Saunders et al. 1989).

All of these criteria indicate that some of the conditions at which we reared *H. hebetor* resulted in adult reproductive diapause. Ovarian development was reduced when *H. hebetor* females were reared during the immature stages at 17.5 and 20°C, and there was little ovarian development when females were reared with a 10-h photoperiod at these low temperatures. Rearing *H. hebetor* at 17.5 and 20°C did not result in diapause of immature stages (no evidence of unusually prolonged immature developmental times), but did appear to result in possible adult reproductive diapause. Females reared during the immature stages at 17.5°C with a10-h photoperiod lived longer and took longer to lay their first eggs and to lay 50% of their eggs than those females reared at 17.5°C with a16-h photoperiod. Females reared during the immature stages at 20°C with a10-h photoperiod took longer to lay their first eggs and to lay 50% of their eggs, and they had a lower respiration rate, than those females reared at 20°C with a16-h photoperiod. Females that were reared during the immature stages at conditions that appeared to induce reproductive diapause resumed ovarian development and oviposition, and their respiration rate was three times higher, after transfer to a higher temperature (27.5°C). Thus, females reared during the immature stages with a 10-h photoperiod at 17.5 and 20°C appeared to enter reproductive diapause.

Doutt (1959) hypothesized that a parasitoid may enter diapause for synchronization of development between the host and the parasitoid. Flanders (1944) hypothesized that the purpose of diapause in parasitoids is to delay development so that the host is at the stage that is most suitable for the parasitoid. It seems likely that *H. hebetor* may enter diapause to synchronize its development with that of its host, *P. interpunctella*, which overwinter as diapausing last instars (Carrillo et al. 2006). The diapausing host larvae terminate diapause in the spring and then pupate. Therefore, if *H. hebetor* were to overwinter as diapausing larvae or pupae, then they would not be able to find *P. interpunctella* larvae for parasitization after diapause termination and emergence in the spring. However, when *H. hebetor* overwinter as adults in reproductive

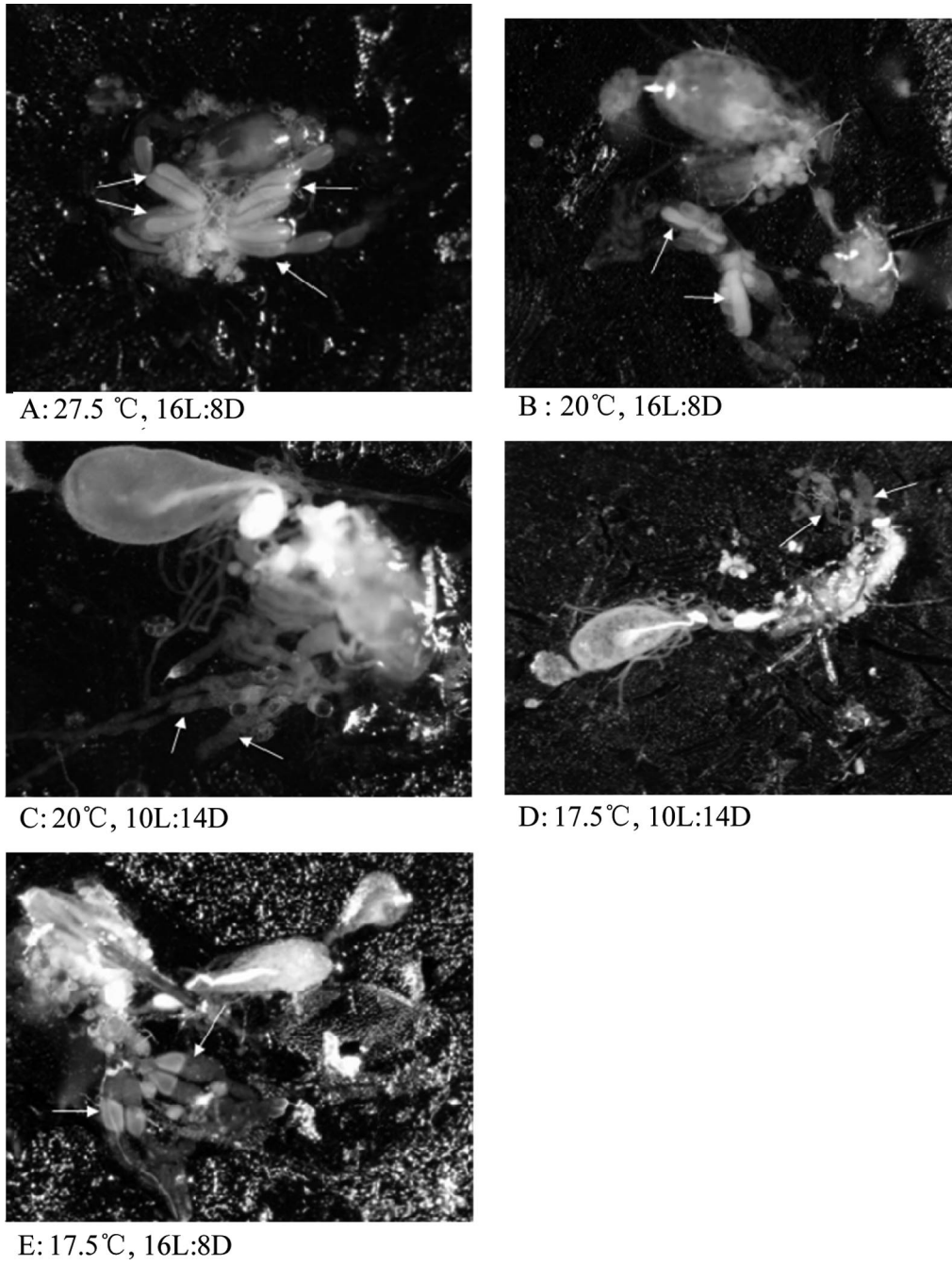

Fig. 2. Ovaries of *H. hebetor* females reared at different photoperiods and temperatures after a 3.5–7.0-d postemergence period (ovaries are indicated by arrow). (Online figure in color.)

diapause, they are ready to find and parasitize *P. interpunctella* larvae in the spring.

The fat body has an essential role for the insect in an unfavorable environment because most of the insect's intermediary metabolism takes place in this organ, including lipid, carbohydrate, amino acid, and nitrogen metabolism, as well as protein synthesis. The fat body also serves as an organ for energy storage and utilization (Haunerland and Shirk 1995, Arrese and Soulages 2010). Most of the diapause-associated proteins are synthesized in the fat body (Denlinger 2002). Some of those diapause-associated proteins are synthesized before the onset of diapause, and those specific proteins are released into the hemolymph and remain there throughout diapause (Brown and Chippendale 1978, Brown 1980, Sula et al. 1995, Godlewski

et al. 2001). The abdomen of female *H. hebetor* reared during the immature stages at 17.5°C was filled with the fat body, which may be how *H. hebetor* prepare to overwinter or to survive in unfavorable environments.

Diapausing larvae of the midge *Aphidoletes aphidimyza* (Rondani), which feed on aphids, could be stored in their cocoons for 3 mo at a low temperature (4°C) with a rate of mortality of ≈25%, but 50% of nondiapausing larvae died after 1 mo in the same conditions (Havelka 1980). The emergence rate from diapausing pupae of the braconid parasitoid *Microplitis mediator* (Haliday) was 61.7% after 360 d storage at 4°C (Hun et al. 2005). Our data show that reproductive diapause is likely induced in *H. hebetor* when reared during the immature stages with a 10-h photoperiod at 17.5 and 20°C, but whether these adults can be stored longer than nondiapusing adults and whether their performance after storage is impacted by cold storage remains to be determined.

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