Physiological Entomology (1989) **14**, 361–367

Simulated winter to summer transition in diapausing adults of the lady beetle (*Hippodamia convergens*): supercooling point is not indicative of cold-hardiness

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ABSTRACT. Adults of *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) form massive overwintering aggregations in the Sierra Nevada Mountains of California. These diapausing pre-reproductive adults may remain in the aggregations for up to 10 months. Beetles were collected from overwintering sites in Nevada County, California, and held at 4°C for 6 weeks. In order to simulate the winter to summer transition, the beetles were transferred to 20°C and 68% r.h. under a LD 12:12 h cycle. Supercooling points increased slightly from −16°C on day 0 to −12.9°C on day 21. In contrast, low temperature tolerance decreased markedly within 2 weeks. On day 0 nearly all beetles survive 2 h of exposure to −5°C, whereas only 50% survived this treatment on day 4. These data demonstrate the general lack of correlation between the supercooling point and the lower lethal temperature in warm-acclimated beetles. Within 1 day of transfer to 20°C, oxygen consumption decreased by 26%. This decrease continued through day 14 when the rate of oxygen consumption had decreased to 40% of initial values. Beetles acclimated to 20°C selected temperatures significantly higher than cold-acclimated beetles. The parametric shifts observed in this study are consistent with a physiological transition from cold-hardiness and energy conservation during winter to one of dispersal, feeding and reproduction in summer beetles.

Key words. Diapause, cold-hardiness, acclimation, Coccinellidae, supercooling point.

Introduction

Insect dormancy or diapause functions to coordinate insect life cycles with phenological changes in the environment (Tauber *et al.*, 1986; Danks, 1987). Entry into this state is a programmed response usually characterized by a cessation of feeding, a depressed metabolic rate and a lack of morphogenesis. Dormancy allows insects to survive environmental periods of drought, high and low temperatures as well as seasonally available food sources. Traditionally, diapause is considered to be a relatively fixed and unresponsive state incapable of physiological compensation to changing environmental conditions; more recently, it has been recognized that diapause is better considered a dynamic and regulated state (Tauber *et al.*, 1986; Danks, 1987).

A number of recent reviews focus on

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metabolic and enzymatic adaptations to temperature in ectotherms (Hochochka & Somero, 1984; Hoffman, 1985; Cossins & Bowler, 1987). Most studies have examined the potential for compensatory acclimation of parameters, such as heart rate, the activity of digestive enzymes or locomotion, that function to maintain an energetically active state; while relatively few investigations have examined this capacity in dormant or inactive stages that would be expected to minimize the use of energy reserves.

Although diapause and cold-hardiness are often closely linked temporally, the precise relationship between these physiological states remains unclear. In some species such as the flesh fly, *Sarcophaga crassipalpis*, and the rice stem borer, *Chilo suppressalis*, these states appear to be firmly linked (Adedokun & Denlinger, 1984; Lee & Denlinger, 1985; Tsumuki & Kanehisa, 1978). In contrast, the beetle *Dendroides canadensis* exhibits the capacity to cold-harden, but lacks the capacity to enter diapause (Horwath & Duman, 1983). In yet another case, diapausing larvae of the European corn borer lack the capacity to cold-harden during the summer months, while acquiring cold tolerance during the autumn (Hanec & Beck, 1960). Relatively few studies have simultaneously examined the environmental factors and physiological processes that regulate the transition from overwintering diapause and cold-hardiness to those associated with the active periods of feeding and reproduction during the summer.

The convergent lady beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), is distributed widely in North America and is well known for its habit of forming, sometimes massive, overwintering aggregations. One report describes an aggregation from which more than 40 million beetles were collected (Hagen, 1962). In the spring California populations of *H.convergens* migrate from the lowlands to the Sierra Nevada Mountains where the aggregations are formed (Hagen, 1962). The release of an attractant pheromone aids in the maintenance and structure of these aggregations (Copp, 1983). These diapausing pre-reproductive adults remain in the overwintering aggregations for up to 10 months before returning to the lowlands. This extended dormancy period functions to synchronize the feeding and reproductive activities of this univoltine species with their aphid food supply.

This study simulates the winter to summer transition by acclimating diapausing adults of *H.convergens* to 20°C. In particular, the relationship between the supercooling point and the lower lethal temperature is evaluated. The influence of acclimation to warmer temperatures on the capacity for physiological compensation of respiration rate and temperature preference is described in the context of its significance to overwintering bioenergetics. Efficient utilization of energy reserves is likely of critical importance to this species due to its extended period of dormancy and its inability to replenish its reserves during this time.

Methods

Diapausing adults of *H.convergens* were collected from their mountain overwintering sites near Grass Valley, Nevada County, California, U.S.A., in January 1987, stored at 4°C and shipped to our laboratory the next month. The beetles were held in the dark at 4°C for 6 weeks after arrival. At the beginning of the experiment, the beetles were transferred directly to 20°C, and a LD 12:12 h cycle at 68% r.h. Water was provided throughout the experiment. Samples were removed from the various assays according to the following regime: day 0 and 21 control from 4°C, while the 20°C warm acclimated group was sampled on days 1, 2, 3, 5, 7, 14 and 21.

Supercooling points were determined by placing individuals in close proximity to a copper–constant thermocouple, cooling at *c.* 0.3°C/min and recording the temperature at which the release of the latent heat of fusion was detected using a multi-channel chart recorder. Ten beetles were tested for supercooling points for each treatment group. Low temperature tolerance was measured by holding groups of 95–125 beetles for 2 h at −5°C and determining the percentage of animals alive after a 24 h recovery period at 20°C. Respiration rates were determined for individual beetles ($n = 12$) using a micro-respirometry system described by Lee & Baust (1982). Respiration rates at 20°C were reported in μl/mg live weight/h.

Temperature preference was measured using a temperature gradient constructed from a 76 cm aluminium trough attached to 24 cm brass legs. For each trial, one leg was immersed in a bath at −10°C while the other remained at room temperature (25°C). The resulting temperature in the gradient ranged from 4°C to 21°C. Using a temperature gradient of this type it is difficult to prevent the development of a corresponding moisture gradient. However, we did not observe condensation of water droplets anywhere in the gradient during our experiments. Groups of fifty insects were placed in the centre of the gradient and their position recorded 2 h later. Water content was determined by drying the beetles to a constant weight at 50°C. Means were compared using Student's two-tailed *t*-test. Clumping behaviour was analysed using the methods of Elliott (1983).

Cryoprotectant analysis utilized high performance liquid chromatography (HPLC) techniques as described previously (Baust *et al.*, 1983; Lee *et al.*, 1987b). Groups of ten to fifteen beetles with a total weight of approximately 200 mg were homogenized with a polytron tissue homogenizer in 3 ml of ethanol. An internal standard of xylose was used to determine percentage recovery. The homogenate was centrifuged at 2000 *g* for 5 min, the pellet re-extracted twice with ethanol and re-centrifuged. The combined supernatants were passed through a Sep-Pak C18 cartridge and evaporated to dryness. The sample was resuspended in 75% ethanol and filtered through a 0.22 μm membrane filter prior to HPLC analysis. This system uses a radially compressed silica column with a recirculating mobile phase of acetonitrile : water : tetraethylenepentamine (81 : 19 : 0.02).

Results

Supercooling point and lower lethal temperature

Supercooling points increased significantly ($P < 0.05$) from a mean value of −16.0°C on day 0 to −12.9°C on day 21 (Fig. 1). Even on day 2, supercooling points were significantly higher than on day 0. By day 7 supercooling point values reached a plateau near −13°C, where it remained through the last day of the experiment. Supercooling points remained unchanged from initial values for a control group of beetles held at 4°C throughout the 21-day experiment. No individual survived at temperatures below the supercooling point.

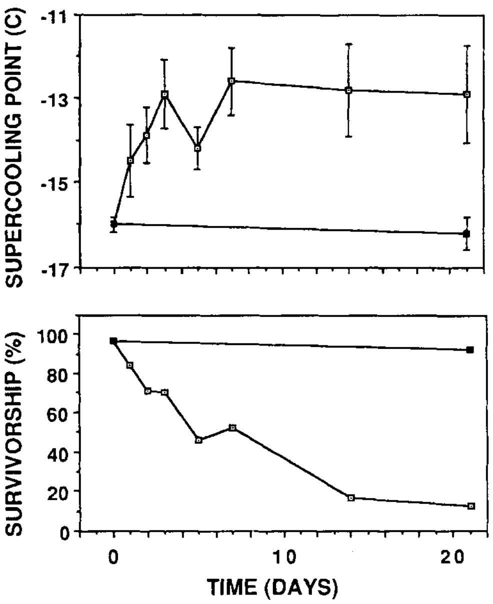

FIG. 1. The effect of acclimation to 20°C on the supercooling point (mean ±SEM, $n=10$) and low temperature tolerance in diapausing adults of *Hippodamia convergens*. Low temperature tolerance was measured as the proportion of individuals surviving at 2 h exposure at −5°C (mean ±SEM, $n=95–125$). Solid symbols represent controls maintained at 4°C, while open symbols correspond to beetles acclimated to 20°C.

The slight increase in the supercooling point with warm acclimation occurs in marked contrast to the dramatic decrease in low temperature tolerance observed over the same period (Fig. 1). On the first day of the experiment 96.6% of the beetles survived exposure to −5°C, whereas only 12.9% survived after 3 weeks of acclimation to 20°C. By day 4, low temperature tolerance had decreased to approximately 50% survival at −5°C. Day 21 control beetles retained tolerance comparable to day 0 insects.

Only during the first 3 days is there a correlation between the loss in supercooling capacity and the decrease in low temperature tolerance (Fig. 1). Thereafter, the supercooling point remains relatively constant, while the low temperature tolerance continues to decrease for the duration of the study. There is thus a general lack of correlation between the supercooling point and the lower lethal temperature.

Physiological compensation of respiration rate

Shortly after individual beetles were put into separate respirometers they ceased movement and remained inactive during the 2 h interval in which oxygen consumption was measured. Therefore, we believe that these measurements represent basal or standard metabolic rates of an animal at rest.

The highest respiration rates (1.13 µl O_2/mg/h) were observed on day 0 after direct transfer from 4°C to the measurement temperature of 20°C (Fig. 2). Within 1 day of acclimation to 20°C the respiration rate decreased by 26% to 0.83 µl O_2/mg/h. Respiration rates continued to decrease until day 14 where values plateaued until day 21. This plateau corresponded to nearly a 60% reduction in respiration rate as compared with the initial value.

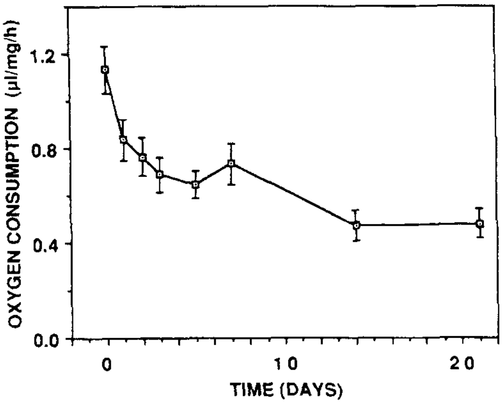

FIG. 2. The effect of acclimation to 20°C on the respiration rate at 20°C of diapausing adults of *Hippodamia convergens* (mean ±SEM, $n = 12$).

Live weight and water content

There was no significant change in live weight from day 0 to day 21 (approximately 14 mg/beetle), however, there was a significant increase ($P < 0.05$) in water content between these two days (58% on day 0 *v*. 64% on day 21).

Temperature preference

Groups of forty-five to fifty beetles were placed in the temperature gradient periodically during the 21-day acclimation period. On day 0 directly after transfer from 4°C, the majority of the beetles selected the cold end of the gradient with a mean selected temperature of 6.9°C (Fig. 3). Only a few individuals selected temperatures above 12°C, with 68% of the population in the 4–6°C range. Within 1 day of transfer to 20°C the mean preferred temperature increased by approximately 3.5°C. Between days 1 and 7 the average selected temperature was between 10 and 14°C. Between days 7 and 14 a marked change in temperature preference occurred with most beetles selecting temperatures near 18°C. On days 14 and 21 no beetle selected a temperature below 11.9°C. By day 21 the majority of beetles selected the warm end of the gradient with a mean of 18.4°C (Fig. 3).

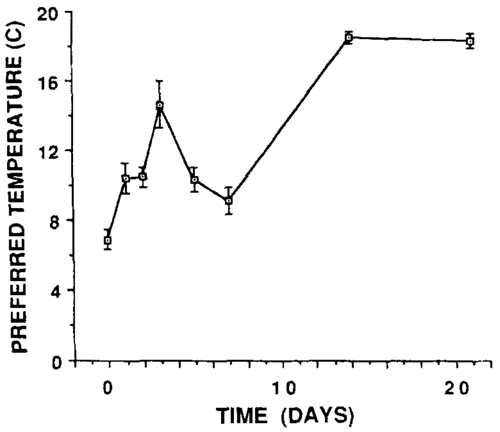

FIG. 3. The effect of varying the duration of acclimation to 20°C on the temperature preference of adults of *Hippodamia convergens*.

On day 0 beetles were distributed in the gradient in clusters of ten to fifteen individuals (Fig. 4). A similar pattern of clumping was observed on day 5. Within the first 7 days of transfer to 20°C the sample variance was substantially greater than the sample mean. According to Elliott (1983), this represents an aggregated distribution and can be described by a negative binomial model. On days 14 and 21, the small clusters of beetles were no longer apparent. Furthermore, on these days the sample variance was substantially less than the mean, indicating a loss of the aggregated distribution.

Cryoprotectant screening

In tissue extracts from the adult beetles, none of the commonly reported cryoprotectants including glycerol, sorbitol, trehalose or mannitol were identified using HPLC analysis in concentrations greater than 1 mM.

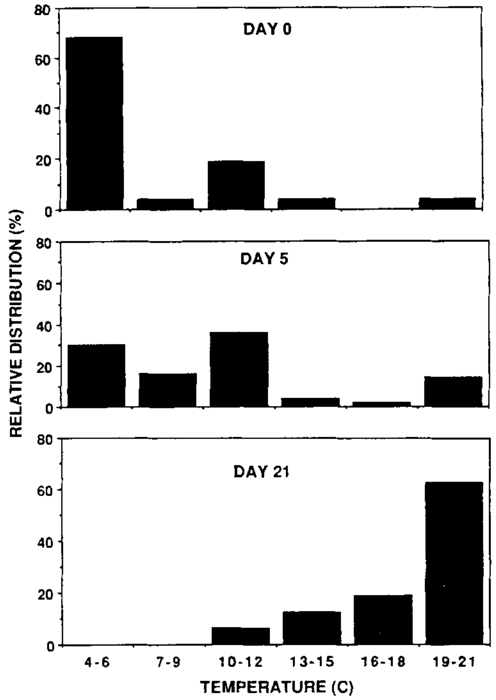

FIG. 4. Distribution of *H.convergens* in a temperature gradient after direct transfer from 4°C (top, Day 0) and 5 and 21 days of acclimation to 20°C.

Discussion

Exposure to low temperature is the primary environmental cue triggering both cold-hardening and cryoprotectant accumulation in insects (Baust, 1982; Baust & Lee, 1982; Zachariassen, 1985). Temperature typically plays a secondary, and sometimes interactive, role to that of photoperiod in programming diapause (Beck, 1980; Saunders, 1982). The entry of coccinellids into adult diapause is influenced by both photoperiodic and temperature cues (Hodek, 1973; Tadmor & Applebaum, 1971; Storch & Vaundell, 1972). Exposure to high temperature has frequently been identified as a factor contributing to the loss of cold-hardiness and the termination of diapause.

The exposure of overwintering insects to high temperatures results frequently in a rapid loss of glycerol and other polyols, and a loss of low temperature tolerance (Dubach *et al.*, 1959; Somme, 1964; Baust & Miller, 1972). In the lady beetle, *Coleomegilla maculata*, exposure to 25°C results in a rapid loss of cold-hardiness and an elevation of the supercooling point within 2 days (Baust & Morrissey, 1975; Lee, 1980). In contrast, the supercooling point remained essentially constant at approximately −17°C for diapausing adults of *H.convergens* held at 25°C for 1 month (Lee, 1980). A similar response was observed in the present study, except that the supercooling point increased gradually from −16°C on day 0 to −13°C on day 3 where they remain for the rest of the 21-day acclimation period.

The results of this study demonstrate clearly that the supercooling point is not an adequate measure of the lower lethal temperature. On day 0 virtually all individuals tolerate 2 h of exposure to −5°C; however, within 1 day of transfer to 20°C a decrease in low temperature tolerance is observed. On day 7, only 50% of the beetles survive 2 h at −5°C and only 12.9% on day 21. Thus, cold-hardiness continues to decrease steadily throughout the acclimation period, despite the fact that supercooling points remained constant near −13°C from day 3 to day 21.

Several other recent investigations have demonstrated the unreliability of the supercooling point as a measure of low temperature tolerance (Turnock *et al.*, 1983; Lee & Denlinger, 1985; Lee *et al.*, 1987a, b; Bale, 1987). In the flesh fly, *Sarcophaga crassipalpis*, ontogenetic patterns of supercooling point profiles are similar within larval, pupal and adult stages regardless of whether the flies are programmed to enter diapause (Lee & Denlinger, 1985). The overwintering diapause pupae survives temperatures approaching its supercooling point of −23°C, while non-diapause pupae with the same supercooling point do not. In this species the supercooling point is a reliable indicator of low temperature tolerance only during the latter stages of the pupal diapause. The grain aphid experiences 50% mortality at −8.1°C even though its supercooling point is −24.2°C (Knight *et al.*, 1986).

The decrease in the rate of oxygen consumption upon warm acclimation is consistent with previous findings for this species (Lee, 1980). This type of compensatory acclimation is classed as Type I, excess or overshoot compensation, according to Precht (1958). The terms 'excess and overshoot' were originally derived from a comparison of the rate of activity at an initial temperature with that after a period of acclimation to a second, in this case, higher temperature. If, after acclimation, the rate returns to the initial level then compensatory acclimation is said to be complete or, as observed in this study, a decrease in rate as compared to the initial value is categorized as excess or overshoot compensation. In their overwintering aggregations, *H.convergens* are exposed to fluctuating temperatures, sometimes on a daily basis. In view of the 10-month duration of diapause and the inability to replenish their energy reserves during this period, the rapid decrease in their rate of oxygen consumption functions to conserve energy. As a result the terms excess and overshoot seem inappropriate for a dormant or overwintering organism in which mechanisms for the conservation of energy would be of critical importance.

Acclimation to high temperature has a marked effect on the temperature preference in *H.convergens*. Cold-acclimated beetles select low temperatures. A similar positive cryotaxic response is observed in an overwintering arctic carabid (Baust & Miller, 1970). In overwintering beetles this response would function, not only to conserve energy reserves by the direct action (Q_{10} effect) of low temperature on the metabolic rate, but it would also serve to maintain the maximal level of cold tolerance. Under natural conditions sustained exposure to low temperatures over a period of days or weeks would only occur near the end of diapause and may function to signal a shift in physiological state from that of diapause to one of dispersal, feeding and reproduction.

One possibility for enhancing insect cold-hardiness is the synthesis of antifreeze proteins (Duman *et al.*, 1982). Although Duman (1979) reported the presence of thermal hysteresis factors in a coccinellid, studies in our laboratory suggested that these proteins were not present in overwintering adults of *H.convergens* (M. W. Mabry and R. E. Lee, unpublished data).

In general, it appears that coccinellids do not accumulate high concentrations of the commonly reported cryoprotective polyols and sugars (Baust & Morrissey, 1985; Ring & Tesar, 1981; Duman *et al.*, 1982). Hoshikawa (1981) reported the accumulation of inositol ranging between 1.5% and 1.9% of body weight in four species of overwintering coccinellids. In our study of *H.convergens* none of the previously identified cryoprotectants were identified. Additional studies are needed to determine what role, if any, low molecular weight polyhydric alcohols and sugars play in the cold-hardiness of coccinellids.

Acknowledgments

We thank J. S. Bale, A. L. Rypstra and P. K. Westfahl for their comments on an earlier draft of this manuscript. M. W. Mabry provided technical assistance in the early stages of this study and R. A. Dommel prepared the figures. This study was supported by a Grant-In-Aid of Research from Sigma Xi to L.E.B. and a grant from the National Science Foundation, DCB-8517875, to R.E.L.

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Accepted 3 January 1989