

## PHYSIOLOGICAL ADAPTATIONS OF COCCINELLIDAE TO SUPRANIVEAN AND SUBNIVEAN HIBERNACULA

RICHARD E. LEE, JR.\*

Zoology Program, Department of Ecology and Behavioral Biology, University of Minnesota,  
Minneapolis, MN 55455, U.S.A.

(Received 6 June 1979; revised 24 September 1979)

**Abstract**—The supranivean hibernaculum of *Hippodamia convergens* allows exposure of this species to fluctuating temperatures and humidities. Correspondingly, *H. convergens* is resistant to desiccation, maintains cold-hardiness independent of temperature, and responds to warm acclimation with a reduced respiration rate. Beneath the snow, *Coleomegilla maculata* is maintained at a constant temperature and high humidity. Warm acclimation in this species results in a loss of cold-hardiness and an increase in respiration rate. This response suggests a transition from an energy-conserving metabolic state associated with overwintering to one of energy expenditure as the beetles prepare for summer feeding and reproduction.

**Key word index:** Insect cold-hardiness, respiration, acclimation, hibernaculum, Coccinellidae, *Hippodamia convergens*, *Coleomegilla maculata*

### INTRODUCTION

ALTHOUGH a number of workers have studied the physiological and biochemical basis of arthropod overwintering, few investigations have considered the significance of particular hibernacula in relation to overwintering physiology (DANKS, 1978). Exceptions include the comparative studies of KIRCHNER (1973) with spiders and MACPHEE (1964) with orchard arthropods; species from exposed, above ground sites exhibited greater cold-hardiness (i.e. lower supercooling points) than ones from more protected hibernacula.

The lady beetles, *Hippodamia convergens* Guerin and *Coleomegilla maculata lengi* Timberlake differ in their overwintering habits. During May and June, California populations of *H. convergens* migrate from the lowlands to the Sierra Nevada Mountains where aggregations are formed (HAGEN, 1962). The beetles remain in these aggregations until February, then return to the valleys. HAGEN (1962) notes that during the winter these aggregations are generally not covered by snow. LATA (1928) in Iowa, and HODSON (1937) in Minnesota, also, observed overwintering aggregations of *H. convergens* in relatively exposed sites beneath loose bark and in crevices of a fencepost. The aggregations of *C. maculata* in leaf litter along the margins of woods are generally covered by snow (HODSON, 1937; SOLBRECK, 1974). M. WARSHAW (personal communication) indicated that aggregations not covered by snow experienced the greatest mortality through the winter in Vermont.

Although previous workers have demonstrated a reduction in respiration rate during dormancy for *H. convergens* (STEWART *et al.*, 1967) and *C. maculata* (PARKER *et al.*, 1977), no studies have investigated possible temperature compensation of respiration rate for these species.

This report provides comparative data on several physiological parameters for individuals of *H. convergens* and *C. maculata* collected from winter aggregations. These data appear to correlate closely with the environmental conditions found in the specific hibernaculum of each species.

### METHODS AND MATERIALS

#### Collection

During the autumn of 1977 and 1978 several thousand individuals of *C. maculata* were collected from an overwintering aggregation in Winona County in south-eastern Minnesota. Samples of *H. convergens* were collected from aggregation sites near Grass Valley, Nevada County, California. Additional summer samples of individuals of both species were collected from corn fields in central Minnesota.

#### Respiration

Respiration rates were measured using Warburg manometry (UMBREIT *et al.*, 1972). Beetles were acclimated at either  $6 \pm 0.5$  or  $20 \pm 0.5$  °C prior to respiratory rate determinations. The centre well of the 15 ml flasks was covered with a stainless steel screen to prevent contact between the beetles and the 10% potassium hydroxide solution. Beetles were placed in the flasks and allowed to equilibrate for 15 min. The respiration rate of 4–10 beetles was determined for a following two hour period. An initial set of respiration rates was determined over the 0–20 °C range for beetles which had been acclimated to 6 °C for 2 months. A second set of determinations were made after acclimation to 20 °C and 75% r.h. for 5–10 days.

#### Resistance to desiccation

*H. convergens* and *C. maculata* were acclimated to 6 °C for two months. Fifty individuals were then transferred to each of the following combinations of temperature and r.h.: 6 °C, 75% r.h.; 6 °C, 0% r.h.;

\* Present address: Department of Biology, University of Houston, Houston, TX, 77004, U.S.A.

20°C, 75% r.h. and 20°C, 0% r.h. Every three days, the beetles were examined for 15 min at 23–25°C and uncontrolled humidity; mortality was indicated if no movement was observed.

#### Supercooling point

Beetles were acclimatized in screened field cages covered with 15 cm or more of snow for one month prior to supercooling point determinations. The supercooling point of individual beetles was measured to the nearest 0.5°C using a temperature probe (YSI No. 427) and a battery-operated thermometer (YSI Telethermometer). The probe was held against the ventral surface of the beetle. The beetle and probe were then enclosed in a 20 ml stoppered test tube and immersed in an alcohol bath (cooling rate of 3–4°C/min). Supercooling points were determined at the onset of needle deflection to warmer temperatures. This deflection was indicative of the liberation of the latent heat of fusion. Each sample mean is based on 10–15 beetles.

## RESULTS

#### Respiration

As is typical in insect respiration studies, there was an increase in respiration rate as the temperature increased for each of the four respiration–temperature ( $R-T$ ) curves (Figs. 1 and 2). Acclimation to 20°C resulted in a marked shift in the  $R-T$  curve for each species. Respiration rates of *H. convergens* decreased; this is classified as Type I, excess or overshoot compensation by PRECHT (1958). Acclimation of *C. maculata* to 20°C resulted in an increased respiration rate. Precht termed this response, Type 5, inverse or paradoxical acclimation.

#### Resistance to desiccation

*H. convergens* was more resistant to desiccation than *C. maculata* (Fig. 3). Both species had a high rate of survival at 6°C and 75% r.h. This is in agreement with the research of HODSON (1937) and EWERT and CHIANG (1966).

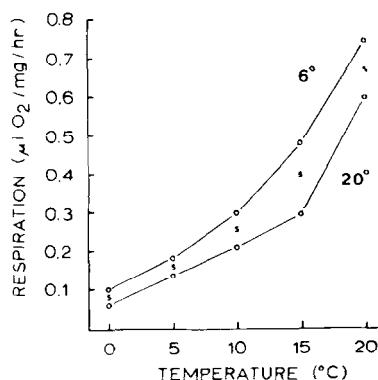


Fig. 1. Effect of temperature on respiration rate for *Hippodamia convergens* acclimated to 6 and 20°C. (s indicates  $p < 0.05$ ).

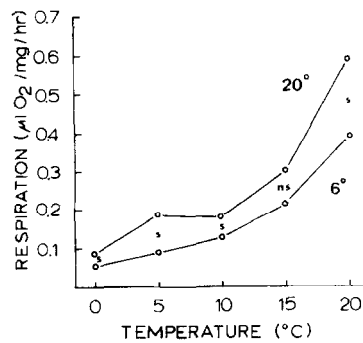


Fig. 2. Effect of temperature on respiration for *Coleomegilla maculata* acclimated to 6 and 20°C. (s indicates  $p < 0.05$ ).

#### Supercooling point

*H. convergens* and *C. maculata* are freezing intolerant, and the supercooling point represents the minimal temperature for survival. Since the probability of freezing is a function of the length of exposure, the supercooling point may occur at higher temperatures in the field (SALT, 1966).

California populations of *H. convergens* were cold-hardy (supercooling point of  $-18.2 \pm 0.7^\circ\text{C}$ ) as compared to July values of  $-7.6 \pm 0.4^\circ\text{C}$  for this species in Minnesota. *C. maculata* undergoes a similar seasonal shift in cold-hardiness with July supercooling points of  $-5.8 \pm 0.3^\circ\text{C}$  decreasing to  $-17.8 \pm 1.0^\circ\text{C}$  in January.

After acclimatization in snow-covered field cages, February supercooling points indicate similar levels of cold-hardiness for both species (Fig. 4). Their responses differed markedly after being held for five days at 25°C and 75% r.h. (Fig. 4). During this period *C. maculata* exhibited an increase, while *H. convergens* maintained a constant and low supercooling point. Even after 28 days at 20°C no increase in supercooling point was observed for *H. convergens*.

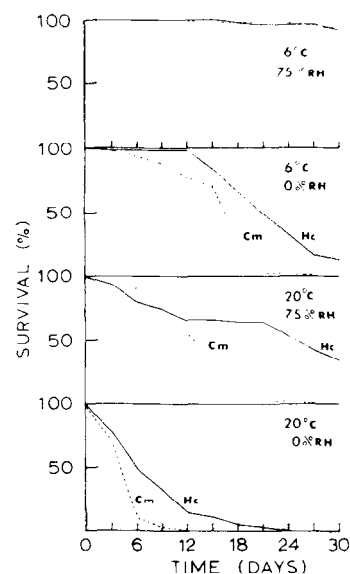


Fig. 3. Survival of *Hippodamia convergens* (solid lines) and *Coleomegilla maculata* (dashed lines) at varying temperature and humidity.

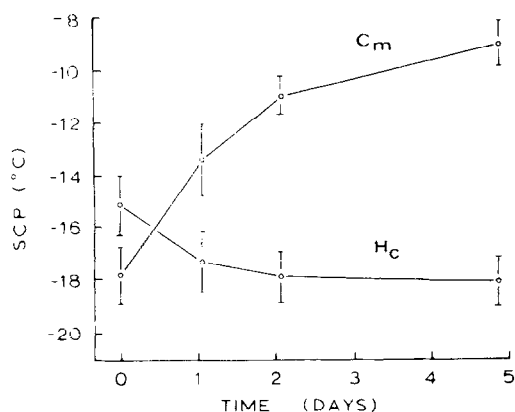


Fig. 4. Effect of acclimation to 25°C and 75% r.h. on supercooling point ( $\bar{X} \pm SE_{\bar{x}}$ ) of *Hippodamia convergens* and *Coleomegilla maculata* (S.C.P.  $\equiv$  supercooling point).

## DISCUSSION

BAUST and MORRISSEY (1975) state that, "Even the most cold tolerant species resident in the Arctic are irreversibly warm-acclimated within hours of above freezing exposures". A number of studies with insects have demonstrated a rapid loss of glycerol, changes in other polyol levels and loss of cold-hardiness after warming (DUBACH *et al.*, 1959; SOMME, 1964; ASHINA, 1969; BAUST and MILLER, 1970, 1972). The rapid increase in supercooling point following warm acclimation in *C. maculata* was originally reported by BAUST and MORRISSEY (1975), and is confirmed by this study.

However, in contrast, warm acclimation in *H. convergens* had no effect on cold-hardiness. H. MANTYLA of the Bio-Control Co. (personal communication) indicated that the mountain aggregation sites from which *H. convergens* was collected were not covered by snow and that the ambient temperatures ranged from  $-7$  to  $10^{\circ}\text{C}$  during the winter. An insect which is exposed to fluctuating temperatures, perhaps on a daily basis, might be expected to have evolved mechanisms for temperature independent retention of cold-hardiness.

It is generally believed that neither compensatory nor non-compensatory acclimation occurs in animals which become dormant or torpid in the cold or warm (PROSSER, 1973, 1975; HAZEL and PROSSER, 1974). However, in this study both non-feeding, reproductively inactive, overwintering lady beetles demonstrated capacity adaptation of respiration. *H. convergens* exhibited reduced respiration rates at all temperatures after acclimation to  $20^{\circ}\text{C}$ . Overshoot compensation of respiration rate has been commonly reported for a number of insects (LUHMANN and DREES, 1952; MARZUSCH, 1952; DEHNEL and SEGAL, 1956; BUFFINGTON, 1969; and NUTTALL, 1970). For an insect exposed to fluctuating temperatures this reduction may result in significant metabolic savings during dormancy when it is not possible to replenish energy reserves. Conversely, warm acclimation in *C. maculata* resulted in increased respiration. Paradoxical acclimation of respiration rate has been rarely reported for insects except for SOMME (1968) and BUFFINGTON (1969).

WIESER (1973) suggested that at least some ectotherms are best considered as 'multistable systems' which respond to environmental changes in ways which are adaptive with respect to the prevailing or 'anticipated' conditions. Under natural conditions *C. maculata* would experience high temperatures only in the spring as the snow melts and the beetles begin to disperse and prepare for summer feeding and reproduction. Thus, considering its subnivean hibernaculum, a reduced metabolic rate in response to lowered temperatures would also result in energy conservation. Further evidence in support of this shift from one stable physiological system to another comes from the work of SOLBRECK (1974) who demonstrated that the maturation of post-hibernation flight behaviour in *C. maculata* was primarily controlled by temperature. The observed loss of cold-hardiness and the increase in respiration are consistent with such a transition.

*Acknowledgements*—I thank Dr. WILLIAM D. SCHMID for his advice and assistance throughout this study. Dr. HUAI C. CHIANG kindly provided equipment and space in his laboratory. Drs. JOHN G. BAUST, HUAI C. CHIANG, and WILLIAM D. SCHMID critically reviewed the manuscript.

## REFERENCES

- ASAHINA E. (1969) Frost resistance in insects. *Adv. Insect Physiol.* **6**, 1–49.
- BAUST J. G. and MILLER L. K. (1970) Variations in glycerol content its influence on cold hardiness in the Alaskan carabid beetle, *Pterostichus brevicornis*. *J. Insect Physiol.* **16**, 979–990.
- BAUST J. G. and MILLER L. K. (1972) Influence of low temperature acclimation on cold hardiness in the beetle, *Pterostichus brevicornis*. *J. Insect Physiol.* **18**, 1935–1947.
- BAUST J. G. and MORRISSEY R. E. (1975) Supercooling phenomenon and water content independence in the overwintering beetle, *Coleomegilla maculata*. *J. Insect Physiol.* **21**, 1751–1754.
- BUFFINGTON J. D. (1969) Temperature acclimation of respiration in *Culex pipiens pipiens* (Diptera: Culicidae) and the influence of seasonal selection. *Comp. Biochem. Physiol.* **30**, 865–878.
- DANKS H. V. (1978) Modes of seasonal adaptation in the insects. I. Winter survival. *Can. Ent.* **110**, 1167–1205.
- DEHNEL P. A. and SEGAL E. (1956) Acclimation of oxygen consumption to temperature in the American cockroach (*Periplaneta americana*). *Biol. Bull. Cambridge Phil. Soc.* **11**, 53–61.
- DUBACH P., SMITH F., PRATT D. and STEWART C. M. (1959) Possible role of glycerol in winter-hardiness of insects. *Nature, Lond.* **184**, 288–289.
- EWERT M. A. and CHIANG H. C. (1966) Effects of some environmental factors on the distribution of three species of Coccinellidae in their microhabitat. In *Ecology of Aphidophagous Insects* (Ed. by HODEK I.) pp. 195–219. Academia, Prague.
- HAGEN K. S. (1962) Biology and ecology of predaceous Coccinellidae. *A. Rev. Ent.* **7**, 289–326.
- HAZEL J. R. and PROSSER C. L. (1974) Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.* **54**, 620–677.
- HODSON A. C. (1937) Some aspects of the role of water in insect hibernation. *Ecol. Monogr.* **7**, 271–315.
- KIRCHNER W. (1973) Ecological aspects of cold resistance in spiders (A comparative study). In *Effects of Temperature on Ectothermic Organisms* (Ed. by WIESER W.) pp. 271–279. Springer, New York.

- LATTA R. (1928) The effect of the extreme temperature of Dec. 7, 8, and 9, 1927 on hibernating *Crioceris asparagi* L. and *Hippodamia convergens* Guer. at Ames, Iowa. *Psyche* **35**, 229-231.
- LUHMANN M. and DREES O. (1952) Über die temperaturabhängigkeit der Atmung sommerschlafender Blattkafer. *Zool. Anz.* **148**, 13-22.
- MARZUSCH K. (1952). Untersuchungen über die Temperaturabhängigkeit von Lebensprozessen bei Insekten unter besonderer Berücksichtigung winterschlafender Kartoffelkafer. *Z. vergl. Physiol.* **34**, 75-92.
- MACPHEE A. W. (1964) Cold-hardiness, habitat, and winter survival of some orchard arthropods in Nova Scotia. *Can. Ent.* **96**, 617-625.
- NUTTALL R. M. (1970) The effect of acclimation upon the survival of *Ptinus tectus* and *Tenebrio molitor* when exposed to low temperatures. *Entomologia exp. appl.* **13**, 217-228.
- PARKER B. L., WHALON M. E. and WARSHAW M. (1977) Respiration and parasitism in *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae). *Ann. ent. Soc. Am.* **70**, 984-987.
- PRECHT H. (1958). Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. In *Physiological Adaptation* (Ed. by PROSSER C. L.) pp. 50-78. Am. Physiol. Soc., Washington D.C.
- PROSSER C. L. (1973) Temperature. In *Comparative Animal Physiology* (Ed. by PROSSER C. L.) 3rd edition pp. 362-428. W. B. Saunders, Philadelphia.
- PROSSER C. L. (1975) Physiological adaptations in animals. In *Physiological Adaptation to the Environment* (Ed. by VERNBERG F. J.) pp. 3-18. Intext Educational, New York.
- SALT R. W. (1966) Effect of cooling rate on freezing temperatures of supercooled insects. *Can. J. Zool.* **44**, 655-659.
- SOLBRECK C. (1974) Maturation of post-hibernation flight behaviour in the coccinellid *Coleomegilla maculata* (DeGeer). *Oecologia* **17**, 265-275.
- SOMME L. (1964) Effects of glycerol on cold-hardiness in insects. *Can. J. Zool.* **42**, 87-101.
- SOMME L. (1968) Acclimation to low temperature in *Tribolium confusum* Duval (Col., Tenebrionidae). *Norsk. ent. Tidsskr.* **15**, 134-136.
- STEWART J. W., WHITCOMB W. H. and BELL K. O. (1967) Estivation studies of the convergent lady beetle in Arkansas. *J. econ. Ent.* **60**, 1730-1735.
- UMBREIT W. W., BURRIS R. H. and STAUFFER J. F. (1972) *Manometric and Biochemical Techniques*. 5th edition. Burgess, Minneapolis.
- WIESER W. 1973. Temperature of ectotherms. A speculative review. In *Effects of Temperature on Ectothermic Organisms* (Ed. by WIESER W.) pp. 1-23. Springer, New York.