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Temporal variation in overwintering gene expression and respiration in the solitary bee *Megachile rotundata*☆

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Abstract

Partial clones of *Megachile rotundata HSP90*, *HSP70*, *HSC70* and *actin* were developed by RT-PCR. These clones were used to generate probes to screen for the expression of their respective transcripts in heat-shocked pupae and in diapausing prepupae through post-diapausing pupae. Northern blot analysis revealed transcript sizes for *MrHSP90*, *MrHSP70*, *MrHSC70*, and *Mractin* of 3.6, 2.3, 2.5, and 1.4 kb, respectively. *MrHSP90* and *MrHSP70* were highly upregulated in post-diapausing pupae exposed to 40 °C for 1 h, while *MrHSC70* was only slightly induced by heat shock. Levels of *MrHSC70* and *MrHSP90* showed little change between field collected diapausing prepupae and post-diapausing pupae. In contrast, *MrHSP70* was highly upregulated in diapausing prepupae and *Mractin* was at or below the level of detection in diapausing prepupae. Transferring field reared overwintering prepupae in February to 25 °C for 3 days induced an expression pattern of *MrHSP70* and *Mractin* more typical of post-diapausing bees, indicating the likelihood that the transition to post-diapause development had occurred prior to February. However, measurements revealed possible cyclic respiration patterns, including low respiratory quotient (RQ) values during February and March and a transition during April to more continuous respiration with elevated RQ values.

Keywords: Insects; Diapause; Heat shock; Actin; Oxygen consumption; Carbon dioxide production; Alfalfa leafcutting bee

1. Introduction

Megachile rotundata (Hymenoptera: Megachilidae) is an adventive, gregarious, cavity-nesting, leaf-cutting bee that has been extensively cultured as a superior pollinator of alfalfa (*Medicago sativa* L.), following the first North American records of its presence (Krombein, 1948; Stephen and Torchio, 1961). At present, *M. rotundata* is the pollinator of choice for alfalfa seed production on more than 70,000 ha in western North America, and is the most widely used commercially managed pollinator after the honey bee, *Apis mellifera* L.

At most North American latitudes, *M. rotundata* emerge and mate during June and July. Nesting continues for 4–6 weeks and nests consist of a linear series of cells constructed from cut-leaf pieces. Each cell is provisioned with a mass of pollen and nectar, on top of which an egg is deposited. Completed nests are sealed with cut-leaf plugs. By mid-summer, fifth instar *M. rotundata* complete the consumption of their pollen-nectar provision, defecate, and spin their cocoon and enter diapause (Stephen and Torchio, 1961; Stephen and Osgood, 1965; Klostermeyer, 1982); "diapause" herein

☆Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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being defined as a refractory period and thus characterized by the inability of the insect to respond to environmental cues, usually elevated temperatures, for subsequent development. Most insects exhibiting diapause display a characteristic U-shaped lifecycle O_2 consumption curve, with the "deepness" of the curve reflecting the apparent "depth" of diapause (Villacorta et al., 1972; Bauman et al., 1978; Tauber et al., 1986; Danks, 1987). In *M. rotundata*, the precipitous late summer drop in respiration levels as non-feeding larvae complete cocoon spinning, and the lack of significant differences in O_2 consumption as prepupae transit from completed cocoon through seven months of wintering, signal the transition from the active prediapause, fifth-instar, spinning-cocoon period, to the diapause induction and maintenance period (Kemp et al., 2004). Most diapausing temperate-zone insect species are in a true diapause condition only during autumn and early winter, and if such individuals are exposed to warm conditions after the winter solstice they will re-initiate development (Tauber et al., 1986; Danks, 1987; Denlinger and Tanaka, 1999).

Chilling greatly accelerates diapause development in *M. rotundata*. Diapausing *M. rotundata* prepupae exhibit a median emergence time of 210–241 days when stored at 31.1 °C versus 28–31 days when chilled at 1.7 °C for 5 months (Johansen and Eves, 1973). Similar results have been reported by Richards et al. (1987) after 4 months of chilling diapausing prepupae at 5–10 °C. In the Logan, UT region, *M. rotundata* should terminate diapause development by late December or early January in most years (Kemp et al., 2004).

In this study, we were interested in whether constant volume respirometry (Kemp et al., 2004) and the expression levels of selected mRNA transcripts, 70 and 90 kDa heat shock protein families and actin, could provide us with a better understanding of the sequential aspects of diapause and post-diapause development in overwintering *M. rotundata* prepupae held under field conditions. A growing body of information demonstrates that the 70 and 90 kDa heat shock protein families are tightly diapause regulated (Yocum et al., 1998; Rinehart and Denlinger, 2000; Rinehart et al., 2000; Denlinger, 2002). The actin cDNA clone, originally developed to serve as an internal control for RNA loading, was discovered to be diapause downregulated and was thus included in this study.

2. Materials and methods

2.1. General approach

Bees used in this study were selected from a population of *M. rotundata* maintained outside under ambient conditions at Logan, UT, USA (Fig. 1). At each sample date, no fewer than 10 individual nests were selected from a nesting block, and individual cells were pooled to minimize the possible influence of sex and within-nest effects. Following methods described in detail below (Sections 2.2–2.8) and in addition to constant volume respirometry (Section 2.9), we conducted a series of four independent albeit sequential experiments involving northern blot analysis, each of

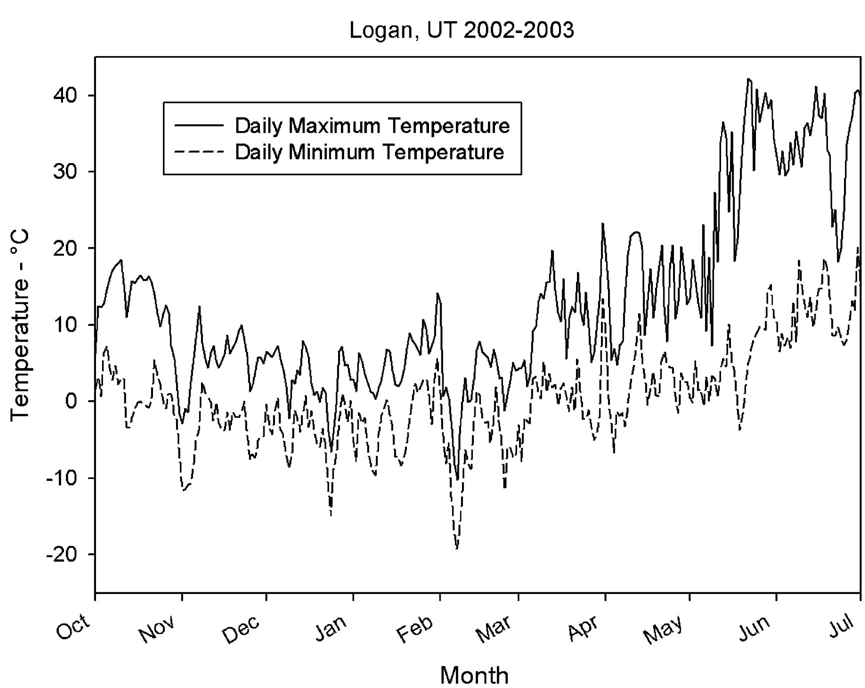

Fig. 1. Daily maximum and minimum temperatures October 2002–July 2003, Logan, UT.