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## Characterization of a Major Hemolymph Protein (MHPB) and its Role in the Ovarian Development of the Lubber Grasshopper

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Poster Presentation P34

**CHARACTERIZATION OF A MAJOR HEMOLYMPH PROTEIN (MHPb)  
AND ITS ROLE IN THE OVARIAN DEVELOPMENT OF  
THE LUBBER GRASSHOPPER**

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Hemolymph in the lubber grasshopper *Romalea microptera* contains high levels (up to 100 mg/ml) of proteins that may be used to store amino acids. SDS-PAGE disclosed three major hemolymph proteins (MHPa1, a2, and b) with sizes of 68, 74, and 83 kDa, respectively. One of these, MHPb, was studied further. Gel filtration HPLC indicated a size of 310 kDa, suggesting that the native protein is a tetramer. MHPb was purified by anion-exchange HPLC and used to generate a rabbit antiserum. Western blots showed one major protein (~80 kDa). MHPb was present in the hemolymph of juveniles and adults. In adult females it accounted for an average of 25% of total hemolymph protein. Vitellogenin (Vg) and MHPb levels were measured using ELISAs. Hemolymph was collected from females fed high or low diets, which caused oviposition at 35 and 65 days, respectively. The average level of MHPb was greater in high-fed ( $12 \pm 1.6$  mg/ml) than in low-fed ( $4.4 \pm 0.6$  mg/ml) animals ( $P < 0.001$ ). In both groups MHPb levels rose to a maximum shortly before the rise in Vg levels that marks the onset of vitellogenesis. This maximum required about 50% more time to occur in low-fed animals. With the increase in Vg levels, MHPb levels dropped rapidly by 50-80%. A similar situation was observed in ovariectomized females. Levels of MHPb reached their maximum prior to the rise in Vg, and then dropped rapidly as Vg levels rose. These data support the hypothesis that MHPb is used to store amino acids that are utilized later, during vitellogenesis. In addition, the levels of MHPb appear to be regulated independent of the ovary. (Supported by NSF grant DBI-9978810 to DWB).