

Physiological Characterization of the Migratory Milkweed Bug, *Oncopeltus fasciatus*

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Introduction

Oncopeltus fasciatus is a migrant colonizer invading northern areas such as Saint Joseph County, Indiana each spring from more southern areas to establish one or more generations during the summer. The population does not overwinter. Some individuals may migrate south in the fall but most are killed by harsh winter conditions (1, 3).

Laboratory cultures of *Oncopeltus* grown at 23°C with a 16 hour photoperiod exhibit long duration tethered flight during the post-teneral prereproductive period. When a 30 minute flight was used to determine migratory organisms, 30% of females and 18% of males of a wild type population were migratory. When these individuals were inbred, the percentage of migratory organisms was increased in the offspring to 60-70% thus demonstrating the genetic basis of this behavior (3).

The ability to fly involves the presence of wings, flight muscles, mitochondria with the ability to produce sufficient ATP and the ability of the fat body to accumulate and release metabolites. A lesion in any part of this chain would lead to a reduced ability to fly. The purpose of this study was to demonstrate that there were several biochemical differences distinguishing the migratory strain from the rest of the population.

Materials and Methods

Rearing: From a wild type laboratory culture of *O. fasciatus* derived from individuals originally collected in Saint Joseph County, Indiana in 1973, a migratory strain and a sedentary strain were selected. Migratory organisms exhibited 30 minute tethered flight during the post-teneral prereproductive period. Sedentary organisms did not. The strains were inbred and annually subjected to additional selection. Colonies were reared in gallon glass jars on milkweed seeds (*Asclepias syriaca*) and water at $23 \pm 3^\circ\text{C}$. Wads of cotton were provided for oviposition sites.

Protein determinations: Protein concentrations of eggs, third instar nymphs, adult males and adult females were determined by the method of Lowry *et al.* (11) after masceration in a Ten Borek tissue grinder in 0.5 N NaOH. Eggs were collected over a 24 hour period for extraction in groups containing between 20 and 85 eggs weighing between 6.5 and 35 mg respectively. Eggs of the migratory strain were derived from pairs of insects each of which had flown for 30 minutes prior to mating and egg laying. Third instar nymphs and adults of both sexes were extracted individually.

Lipid determinations: Lipids were extracted from groups of adult *O. fasciatus* of each strain with a wet weight of between 1 and 5 grams by the method of Folch *et al.* (5). Total lipid samples were evaporated to dryness over steam and under a partial vacuum in a preweighted flask. The flask was then reweighed and the difference recorded as the lipid present in each sample. Lipid content was expressed as the grams of lipid per gram live weight.

Mitochondrial isolation and enzymatic activities: Four thoraces were pooled and homogenized in 2 ml of cold 250 mM sucrose plus 2 mM EDTA (pH 7.4) isolation media. The homogenate was filtered through double layered cheesecloth and the mitochondria were prepared by differential centrifugation using a rapid isolation technique (10). Cytochrome c oxidase activity was measured on a Beckman Dual Beam (DBG) recording spectrophotometer as the rate of decrease in absorbance of reduced cytochrome c at 550nm in a previously described reaction mixture (9). Specific activities were based on the protein content of the mitochondrial samples as determined by the Folin-phenol method of Lowry *et al.* (11).

Results

(Fig. 1) shows the mgs of protein per mg wet weight for eggs, third instar nymphs, adult males and adult females of wild type, migratory and sedentary strains of *O. fasciatus*. No significant differences in protein content were demonstrated in eggs. Third instar nymphs of the migratory strain contained significantly less protein than wild type or sedentary strains. Adult females of the migratory and sedentary strains contained significantly more protein than the wild type strain. Adult males of the migratory strain contained significantly more protein than wild type or sedentary strains.

Table I compares the percent lipid of the wild type, migratory, and sedentary strains of *O. fasciatus*. The wild type strain contained 12.1% lipid; the migratory strain contained 25.4% lipid; the sedentary strain contained 5.4% lipid.

TABLE I Lipid Composition of Wild Type, Migratory, and Sedentary Adult *Oncopeltus fasciatus*

Strain	% Lipid
Wild Type	12.1 (3)
Migratory	25.4 (3)
Sedentary	5.4 (3)

The numbers in parenthesis indicate the number of replications. Each value differs significantly ($P < 0.01$) from the others.

(Fig. 2) shows the activity of cytochrome c oxidase from the flight muscle mitochondria of wild type, migratory and sedentary *O. fasciatus*. Cytochrome c oxidase activity differs significantly and is three fold greater in the migratory strain than in the sedentary or wild type strains.

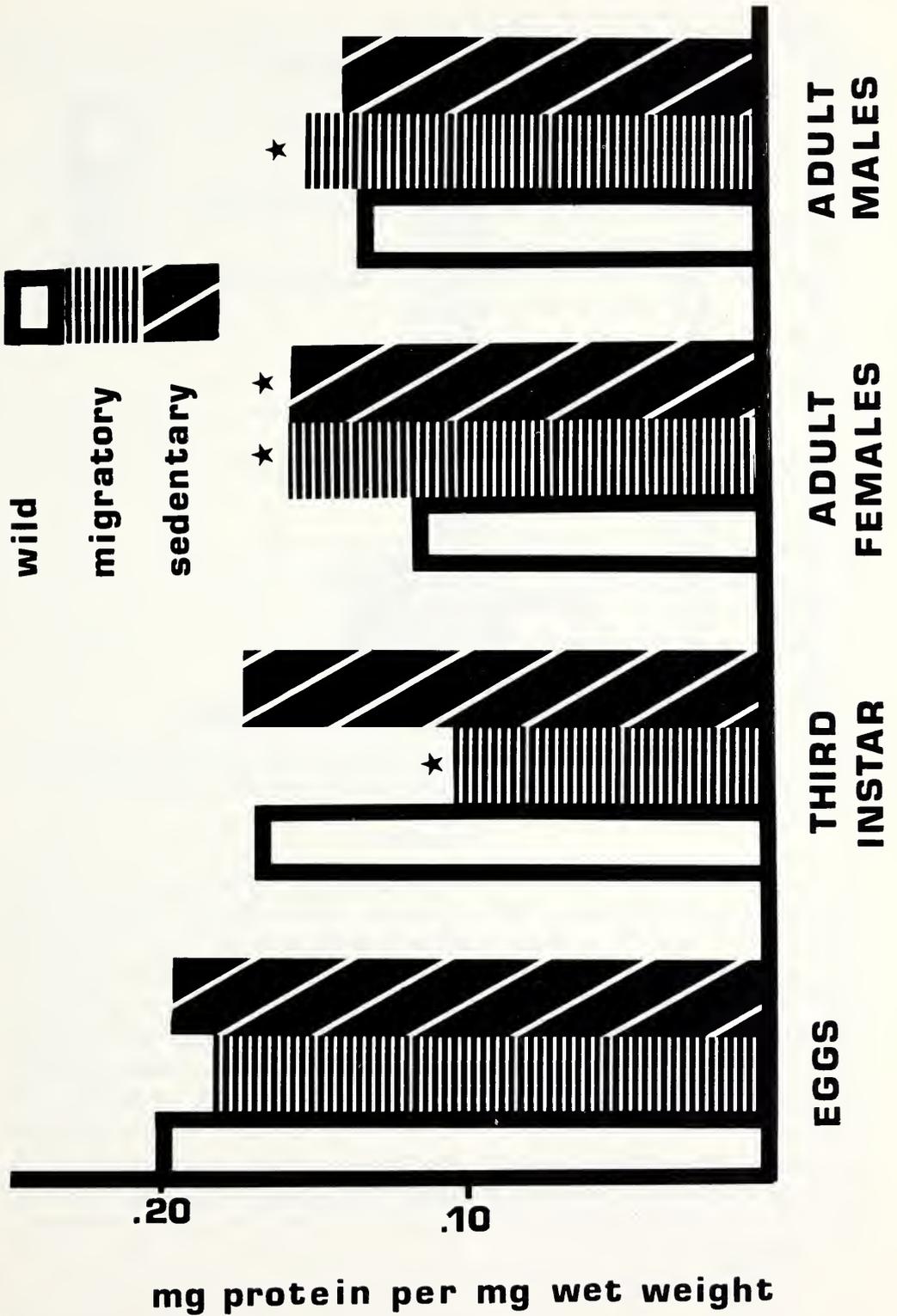


FIGURE 1. Protein concentration of wild type, migratory, and sedentary *Oncopeltus fasciatus* during egg, third instar and adult stages. Each bar represents the mean of 12 determinations. * represents statistical significance.

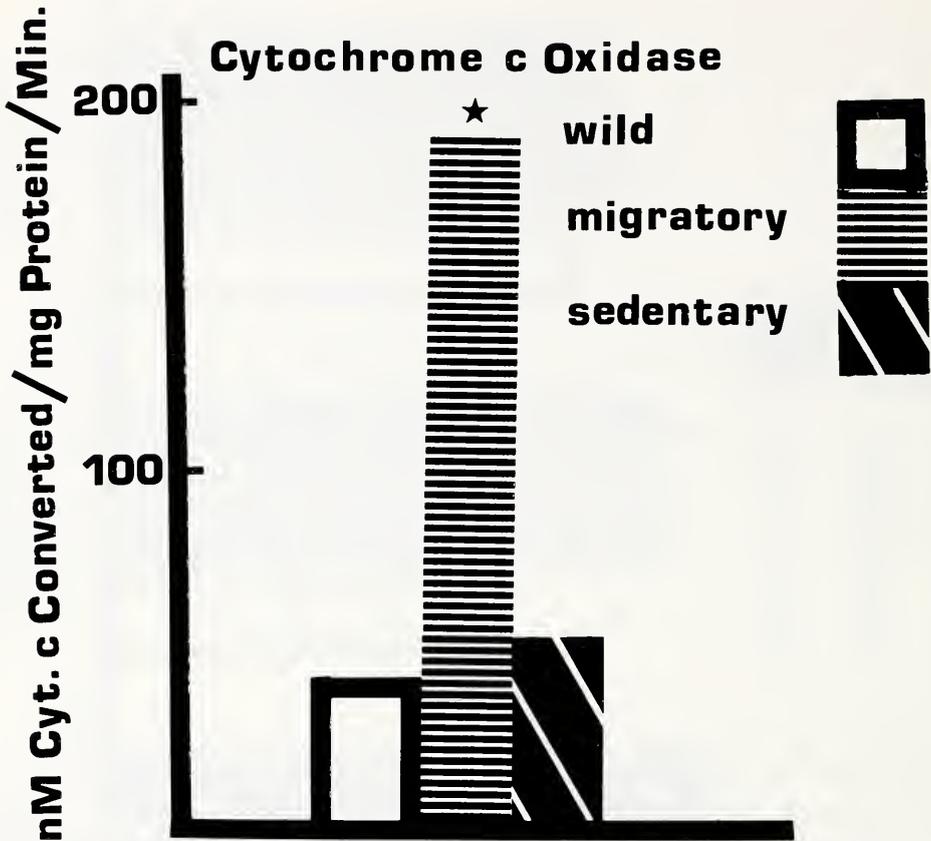


FIGURE 2. Cytochrome *c* oxidase activity in wild type, migratory and sedentary *Oncopeltus fasciatus*. Each bar represents the mean of 4 determinations. ★ represents statistical significance.

Discussion

The results of this study indicate significant differences in total protein, total lipid and cytochrome *c* oxidase activities between wild type, migratory and sedentary strains of *O. fasciatus*. While large numbers of studies have followed protein and lipid composition of insects at different developmental stages (15), relatively few have compared flight forms with flightless stages. The protein concentration of pre-nuptial flight *Solenopsis invicta* decreased 50% during the first 12 hours after flight while lipid concentration remained unchanged (16). The flight form of the adult cowpea weevil, *Callosobruchus maculatus*, had nearly twice as much total body lipid as did the normal weevil (13). These authors did not investigate protein concentration. While earlier studies of *O. fasciatus* characterized the fatty acids of different mutants (6) no studies of the total lipids of migratory and sedentary forms of the milkweed bug are known to these authors.

The studies of flight muscle mitochondrial enzymes can be grouped into three categories: 1, developmental or aging studies in which all the members of the population are considered fliers but not necessarily migrators (7, 8); 2, those comparing a species of normal fliers with another species which is flightless (12); and 3, those describing a totally migratory species (2). Such information must be used carefully when comparing those enzymatic activities to these for migratory strains of *O. fasciatus*. The activity of cytochrome *c* oxidase reported here for the

migratory *O. fasciatus* is the same as that reported for *Heliothis virescens* (8). The general idea that the enzymes of flight muscle mitochondria of fliers differs from non-flying forms is substantiated in this study.

Juvenile hormone has been shown to mediate migratory behavior in *O. fasciatus* primarily through changes in esterase activities destroying the hormone (14). It is unclear at this time how the hormonal changes influence lipid or protein composition and the enzymes of the flight muscle mitochondria. Since there appear to be so many biochemical differences separating the migratory form of *O. fasciatus* from the rest of the population, it is probable that no one simple explanation will be found to explain migration.

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