September 2004

Effect of a Juvenile Hormone Analogue on Lipid Metabolism in a Wing-Polymorphic Cricket: Implications for the Endocrine-Biochemical Bases of Life-History Trade-Offs

Anthony J. Zera
University of Nebraska - Lincoln, azera1@unl.edu

Zhangwu Zhao
University of Nebraska - Lincoln

Effect of a Juvenile Hormone Analogue on Lipid Metabolism in a Wing-Polymorphic Cricket: Implications for the Endocrine-Biochemical Bases of Life-History Trade-Offs

Anthony J. Zera[*]
Zhangwu Zhao
School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588

Accepted 9/12/03

ABSTRACT

The wing-polymorphic cricket, *Gryllus firmus*, has a flight-capable morph (LW[f]: long winged with functional flight muscles) and a flightless morph (SW: short winged with reduced nonfunctional flight muscles) that differ genetically in many aspects of lipid metabolism. To determine whether these differences result from genetically based alterations in endocrine regulation, the juvenile hormone mimic, methoprene, was applied to the LW(f) morph. This hormone manipulation converted the LW(f) morph into a SW phenocopy with respect to all aspects of lipid metabolism studied; that is, methoprene application decreased in vivo biosynthesis of total lipid and triglyceride, increased absolute and relative biosynthesis of phospholipid, increased oxidation of fatty acids, and decreased in vitro specific activities of each of six lipogenic enzymes and a transaminase. Furthermore, methoprene increased ovarian growth and decreased fat body mass and flight muscle mass in the LW(f) morph. Differences in each of these biochemical, morphological, or reproductive traits between hormone-treated and control LW(f) females were similar in magnitude to differences between unmanipulated LW(f) and SW females. Variation in endocrine regulation contributes significantly to genetically based differences in lipid metabolism between LW(f) and SW females. This is the first evidence for endocrine regulation of a genetically based life-history trade-off operating via hormonal effects on specific metabolic pathways and enzymes of intermediary metabolism.

[*] Corresponding author; e-mail: azera1@unlnotes.unl.edu.

Introduction

Life-history traits such as age-specific fecundity and longevity are complex phenotypes that consist of many interacting molecular, biochemical, and physiological components. For decades, evolutionary biologists and ecologists have investigated the physiological (mainly energetic) correlates of life-history variation and trade-offs (Fisher 1930; Townsend and Calow 1981; Rose and Bradley 1998; Ketterson and Nolan 1999; Williams and Vezina 2001; Zera and Harshman 2001; Zhao and Zera 2002). One of the best-studied aspects of this problem has been the functional relationship between energy reserves and life-history traits (Service 1987; Djawdan et al. 1996; Ricklefs 1996; Doughty and Shine 1998; Harshman and Schmidt 1998; Rose and Bradley 1998; Zera and Harshman 2001). Numerous studies have shown that energy reserves such as triglyceride or glycogen often strongly and positively covary with individual life-history traits such as longevity, stress resistance, or dispersal capability. Moreover, reserves covary negatively with other life-history traits (e.g., early age fecundity) that trade off with those mentioned above. These data imply that modification of lipid and carbohydrate accumulation is an important component of life-history evolution.

Much less is known about the biochemical-metabolic causes of life-history variation and trade-offs. For example, very little is known about the extent to which life-history phenotypes differ in activities of enzymes of intermediary metabolism or flux through metabolic pathways that contribute to accumulation of energy reserves (Zera and Harshman 2001; Zhao and Zera 2001, 2002; O'Brien et al. 2002; Zera and Zhao 2003a, 2003b). Furthermore, intermediary metabolism is tightly regulated by hormones (Downer 1985; Steele 1985; Nijhout 1994; Norris 1997). Thus, variation in the endocrine control of intermediary metabolism is expected to be an important cause of adaptive differences between life-history phenotypes in energy reserve accumulation. Yet almost no data are available on this topic (Zhao and Zera 2002; see "Discussion").

We have been using the wing-polymorphic cricket *Gryllus firmus* as an experimental model to identify the physiological, biochemical, endocrine, and genetic bases of life-history variation and trade-offs (reviewed in Zera and Denno 1997; Zera and Huang 1999; Zera and Harshman 2001; Zhao and Zera 2002; Zera and Zhao 2003a, 2003b). Like other wing-polymorphic insects, *G. firmus* consists of a flight-capable

morph with functional wings and flight muscles (LW[f]) and a flightless morph with shortened wings and reduced non-functional flight muscles (SW). Although flightless, the SW morph exhibits substantially elevated ovarian growth and fecundity during the first week of adulthood. An important aspect of morph specialization for flight versus reproduction is the genetically specified enhanced accumulation of triglyceride in the LW(f) morph (Zera and Larsen 2001). Triglyceride is the main flight fuel in *Gryllus* (Zera et al. 1999). Elevated levels of this energy reserve in LW(f) versus SW females result from large-magnitude genetically based differences between morphs in flux through various pathways of lipid biosynthesis and oxidation and activities of enzymes that comprise these pathways (Zhao and Zera 2001, 2002; Zera and Zhao 2003*a*, 2003*b*; see "Discussion").

In this study, we investigated the extent to which differences in endocrine regulation might underlie morph-specific differences in lipid metabolism and ovarian growth in *G. firmus*. Juvenile hormone (JH) positively regulates key aspects of insect reproduction such as yolk protein biosynthesis and uptake into the eggs and negatively regulates many aspects of lipid biosynthesis, accumulation, and flight capability (Downer 1985; Nijhout 1994; Wyatt and Davey 1996; Zera and Cisper 2001; Zera and Harshman 2001). Because of these pleiotropic effects, JH regulation was chosen as the initial focus of our studies on the hormonal regulation of morph-specific differences in intermediary metabolism.

Methoprene is a JH agonist that has been used extensively in insect endocrinology to investigate JH regulation of morphology, reproduction, development, and metabolism (e.g., Dhadialla and Wyatt 1981; Smith and Nijhout 1981; Riddiford 1985; Zera and Tiebel 1988; Wyatt and Davey 1996; and references therein). Methoprene is much more resistant than JH to in vivo degradation, and it is a highly potent and long-acting JH agonist (Zera and Tiebel 1988 and references therein). In this study, we used methoprene to investigate the extent to which activities of enzymes of lipid biosynthesis, rates of lipid biosynthesis and oxidation, and masses of organs involved in lipid metabolism, reproduction, and flight differ between LW(f) and SW morphs due to variation in endocrine regulation. Lipid metabolism can be strongly affected by aspects of an organism's diet (e.g., concentration of carbohydrate and total usable calories; Geer and Laurie-Ahlberg 1984; Downer 1985). To determine the extent to which effects of methoprene on lipid metabolism were contingent on specific dietary conditions, methoprene studies were undertaken on crickets raised on three diets that differed in carbohydrate concentration and total calories.

Methods

Stocks, Morph Characteristics, and Juvenile Hormone Analogue Application

All experiments were conducted on LW(f) females taken from a LW-selected stock of *Gryllus firmus*. This stock is one of several

that has been artificially selected to produce primarily (>90%) the LW(f) morph and has been used in previous biochemical and physiological studies of morph specialization (e.g., Zera and Larsen 2001; Zhao and Zera 2001, 2002; Zera and Zhao 2003*a*, 2003*b*).

In this study, the juvenile hormone agonist methoprene was used to investigate JH-mediated aspects of metabolism. Ten micrograms of methoprene in 2 μL of acetone were applied to the abdomen of LW(f) female *G. firmus* on days 1 and 3 of adulthood. The hydrophobic methoprene is absorbed through the cuticle. Previous studies on *G. firmus* and congeners have shown that this dosage causes ovarian growth in the LW(f) morph that is similar to that observed in unmanipulated SW females (Zera et al. 1998; Zera and Cisper 2001). Applied methoprene also causes the flight muscles to degenerate, thus causing LW individuals to resemble SW individuals with respect to reduced flight muscle mass (Zera et al. 1998; Zera and Cisper 2001). Various biochemical traits were measured on day 5 of adulthood, a point in adult development at which reproductive, morphological, and biochemical traits typically differ to a large degree between morphs (Zera and Larsen 2001; Zhao and Zera 2001, 2002; Zera and Zhao 2003*a*, 2003*b*). In all experiments, control crickets that received neither methoprene nor solvent ("full control") did not differ in any variable under study from the control crickets that received solvent alone (acetone) but not methoprene ("solvent control"). Thus, in statistical tests and data presentation, data from the two control groups were pooled.

Diets and Rearing

All *G. firmus* were raised under standard conditions until adulthood: 28°C, 16L : 8D photoperiod, at a density of 80 and 40 crickets per 10-gal aquarium during the penultimate and last stadium, respectively (see Zera and Cisper [2001] and Zhao and Zera [2001] for additional details). Crickets were fed the standard dry diet up to the penultimate stadium and were fed the "high" diet (same components as the standard dry diet but made up in 2.5% aqueous agar) until the molt to adulthood. After this time, crickets were fed one of three diets: (1) the "high" diet described above, (2) a low-nutrient diet ("low"), in which 75% of the dry components of the high diet were replaced with nondigestible cellulose, or (3) a high-sucrose ("sucrose") diet in which 67% of the cellulose of the low diet was replaced with sucrose. Previous morph-specific aspects of lipid metabolism have been measured on these same three diets (Zera and Larsen 2001; Zhao and Zera 2001, 2002; Zera and Zhao 2003*a*, 2003*b*). Studies were conducted on these three diets to determine the degree to which morph-specific aspects of lipid metabolism were contingent upon specific dietary conditions.

The effect of methoprene on total body, fat body, and ovarian wet mass was determined by dissecting and weighing each of

these organs or whole crickets as described previously (Zera and Cisper 2001; Zera and Zhao 2003*a*). In the case of fat body, only thoracic and abdominal fat body mass (majority of whole-organism fat bodies) was quantified because of the difficulty in obtaining fat bodies from the head and legs. Wet mass of both ovaries was quantified. Flight muscle color (pink vs. white) of the dorsolongitudinal and dorsoventral muscles also was recorded. Flight muscles in *G. firmus* are polymorphic (large, pink, functional vs. small [60% mass of large], white, non-functional), and after the first day of adulthood, color is a perfect indicator of muscle phenotype (Zera et al. 1997; Zera and Cisper 2001).

Lipid Biosynthesis and Oxidation

Rates of lipid biosynthesis and oxidation were quantified in hormone-treated and control *G. firmus* by standard procedures (Downer 1985), as described by Zhao and Zera (2002), Zera and Zhao (2003*b*), and below. Briefly, experiments quantified the amount of radiolabeled [^{14}C]-acetate or [^{14}C]-palmitic acid injected into crickets that was incorporated into various lipid classes (almost exclusively triglyceride and phospholipid) or oxidized to produce CO_2 over a standard period of time. Incorporation of ^{14}C acetate measures the total de novo production of fatty acids and their relative incorporation into triglyceride versus phospholipid, while incorporation of [^{14}C]-palmitate primarily measures the differential incorporation of fatty acid into these two lipid classes (see Fig. 4 of Zhao and Zera 2002). Crickets were fasted for 4 h before injection with radiolabel and were then kept individually in sealed glass containers with paper wicks saturated with an aqueous NaOH solution to trap expired CO_2 (see Zera and Zhao 2003*b*). After an 8-h (acetate injection) or 4-h (palmitate injection) incubation period at 28°C, crickets were homogenized in chloroform/methanol (2 : 1), and triglyceride and total phospholipid classes were separated by column or thin-layer chromatography as described previously (Zhao and Zera 2002). In addition, for [^{14}C]-acetate incorporation studies, the two main biosynthesized phospholipids, phosphatidyl choline and phosphatidyl ethanolamine, were separated by column chromatography as follows: chloroform/methanol (2 : 1) extracts of individual crickets were brought to (4 : 1) chloroform/methanol by the addition of chloroform and were passed through a 0.8-cm^3 silica gel column that had been equilibrated in the same solution. Then 2 mL of the same solution was passed through the column to quantitatively elute triglyceride. Phosphatidyl choline was eluted in 2 mL of 3 : 1 chloroform/methanol, and phosphatidyl ethanolamine was eluted in 4 mL of 100% methanol. The identity of these two phospholipids, which are the main phospholipids found in insects (Downer 1985), was verified by comparison of their rf values relative mobilities on single and two-dimensional thin-layer chromatograms (Christie 1982) relative to standards. Radiolabel incorporated into various lipid classes was determined by liquid scintillation spectrometry.

Enzyme Activities

The effect of methoprene on the specific activities of the following enzymes was studied: fatty acid synthase (FAS), ATP-citrate lyase (ACL, E.C. 4.1.3.8), glucose-6-phosphate dehydrogenase (G-6-PDH, E.C. 1.1.1.49), NADP$^+$-dependent isocitrate dehydrogenase (NADP$^+$-IDH, E.C. 1.1.1.42), asparate aminotransferase (AspAT, E.C. 2.6.1.1), 3-hydroxy-CoA dehydrogenase (HOAD, E.C. 1.1.1.35). These enzymes were chosen to represent various pathways of lipid metabolism. For example, FAS is involved in the de novo pathway of lipid biosynthesis; ACL is involved in the conversion of carbohydrate to lipid; NADP$^+$-IDH and G-6-PDH produce NADPH, a necessary cofactor for lipid biosynthesis; HOAD is involved in lipid oxidation; and AAT is involved in the conversion of amino acids into lipid. Details of enzyme assays can be found in studies by Zhao and Zera (2001) and Zera and Zhao (2003*a*). All enzyme activities were measured in homogenates of fat body, the major organ of lipid metabolism. Because of the expense of many of the substrates of these reactions, activities were only measured on the high and sucrose diets, the two diets in which enzyme activities differ the greatest between unmanipulated LW(f) and SW morphs (Zera and Zhao 2003*a*).

Statistical Analyses

Differences between hormone-treated crickets and controls in organ mass, radiolabel incorporated into various lipid classes or CO_2, or enzyme activity were assessed by ANOVA. Differences between hormone-treated crickets and controls in the proportional incorporation of radiolabel into various lipid classes were assessed by ANCOVA. In these ANCOVAs, radiolabel incorporated into a specific lipid class (e.g., phospholipid) was the dependent variable, while radiolabel incorporated into total lipid was the covariate. Associations among rates of biosynthesis of various lipid classes and rate of oxidation of radiolabel to CO_2 were estimated using Spearman correlations. These correlations were performed on percentage of total incorporated radiolabel found in the various lipid classes and CO_2 (i.e., on standardized incorporation). This was done to eliminate spurious positive associations between variables resulting from differences in total incorporation of radiolabel among individuals.

Results

Effect of Methoprene on Whole-Body and Organ Masses

As expected, methoprene strongly affected the masses and functional characteristics of organs of flight and reproduction in young female *Gryllus firmus* (5 d after molt to adulthood). Ovaries were 150%–400% heavier in hormone-treated versus

control crickets on each of the three diets (Fig. 1). By contrast, all methoprene-treated individuals had white (histolyzed) dorsolongitudinal and dorsoventral flight muscles that were substantially reduced in size, while all control crickets had large pink (functional) flight muscles. Finally, the mass of thoracic and abdominal fat bodies was reduced by about 50% in methoprene-treated versus control crickets on the standard and sucrose diets (Fig. 1). Fat body mass did not differ between hormone and control crickets on the low-nutrient diet, a diet on which fat body mass was already very low in controls (Fig. 1). Increased ovarian mass compensated for decreased flight muscle and fat body masses, resulting in no significant difference between morphs in whole-body wet mass on any diet (Fig. 1).

Enzyme Activities

Methoprene strongly reduced specific activities of nearly all fat body enzymes from crickets fed either the standard (high) or high-sucrose (sucrose) diets (Fig. 2; enzymes were not measured on the low-nutrient diet). For example, on each diet, methoprene treatment resulted in an approximately 50% reduction in the specific activities of FAS and ACL, enzymes involved in de novo biosynthesis of fatty acids (Fig. 2). Similarly, methoprene caused a 30%–50% reduction in the specific activities of the NADPH-producing enzymes, G-6-PDH and NADP$^+$-IDH, and a 26%–31% reduction in AspAT, which is involved in the conversion of amino acids into Krebs cycle intermediates. The one notable exception was the lipid-catabolizing enzyme HOAD, whose specific activity did not differ significantly between control and treatment groups on either diet (Fig. 2). No diet × treatment interaction was observed for the activities of any enzyme, thus indicating that the effect of methoprene on enzyme activity was similar on each diet for each enzyme.

Triglyceride and Phospholipid Biosynthesis from [^{14}C]-Acetate

In hormone-treated crickets, a significantly lower amount of injected [^{14}C]-acetate was incorporated into total lipid relative to controls. This was the case for data pooled over all diets or for total lipid on the high or low diets considered separately (Fig. 3, *top*). A nearly significant ($P = 0.09$) similar trend was observed on the high-sucrose (sucrose) diet (Fig. 3, *top*). Reduced biosynthesis of total lipid from acetate in hormone-treated crickets was due primarily to reduced triglyceride biosynthesis (Fig. 3, *middle*). In none of these analyses of total lipid or triglyceride were significant treatment × diet interactions observed. Incorporation of [^{14}C]-acetate into phospholipid, the other major lipid component of adult *Gryllus*, did not differ significantly between control and hormone-treated crickets over all diets or on most diets tested individually (Fig. 3, *bottom*; no treatment × diet interactions observed; ANOVA, $P > 0.1$). Nor did [^{14}C]-acetate incorporation differ between
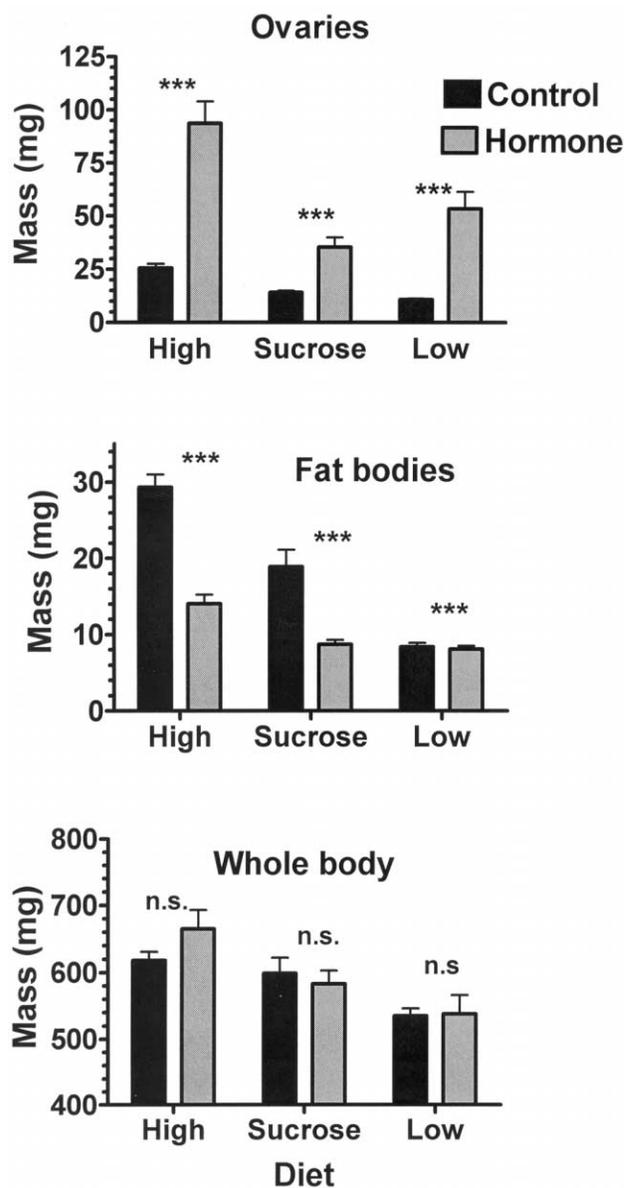

Figure 1. Effect of methoprene on ovarian, fat body, and whole-body wet mass in *Gryllus firmus*. All individuals were long winged and were derived from a long-winged selected line (see "Methods"). Values are means ($\pm$SEM) based on 11–15 (control) or seven to 12 (hormone-treated) crickets. Asterisks above histograms refer to comparisons of means within a diet treatment by ANOVA employing Welsh's correction for unequal variances. *Three asterisks* = $P < 0.005$; *n.s.* = not significant ($P > 0.05$).

treatments for either of the two major phospholipids, phosphatidyl ethanolamine or phosphatidyl choline, when tested on individual diets or pooled across diets (each ANOVA: $P > 0.1$; no diet × treatment interaction; $P > 0.1$, ANOVA). Mean incorporation into phosphatidyl ethanolamine (pooled across diets) was 860 ± 64 dpm ($n = 26$) in controls and 981 ± 69
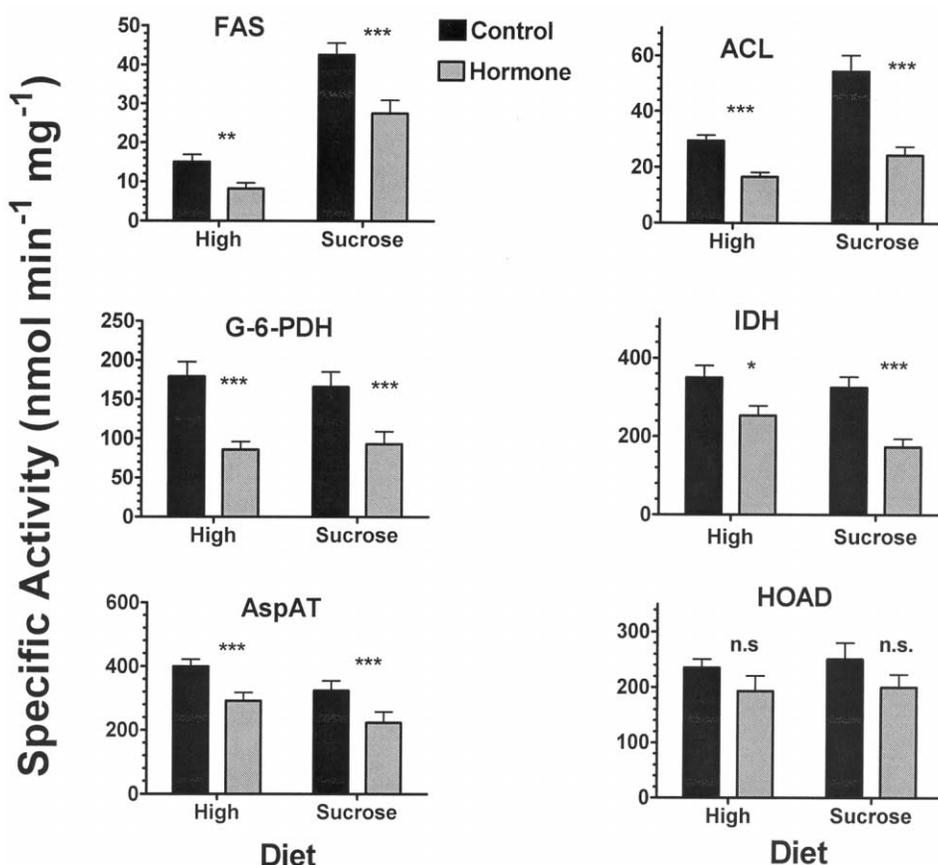
Figure 2. Specific activities (mean ± SEM) of fat body enzymes from control or hormone-treated long-winged *Gryllus firmus*. Means were based on assays of 15–18 (control) or eight to 10 (hormone-treated) individuals. Asterisks above histograms refer to the results of comparisons of means within a diet treatment by ANOVA (*one asterisk* = $P < 0.05$; *two asterisks* = $P < 0.025$; *three asterisks* = $P < 0.005$). In two-way ANOVAs, all enzyme activities differed between control and hormone treatments pooled across diets ($P < 0.005$ in each case), except for HOAD (not significant). All diet × treatment interactions were not significant ($P > 0.05$) in two-way ANOVAs. See "Methods" for composition of diets and full names of enzymes.

dpm ($n = 22$) in hormone-treated females; incorporation into phosphatidyl choline (pooled across diets) was $1{,}247 \pm 89$ dpm ($n = 26$) in controls and $1{,}346 \pm 97$ dpm ($n = 22$) in hormone-treated females.

Results presented above are for absolute incorporation of [^{14}C]-acetate into various lipid components. An important difference between the morphs emerges when relative incorporation of radiolabeled [^{14}C]-acetate into triglyceride or phospholipids was determined. The proportion of total radiolabel incorporated into lipid due to phospholipid was substantially higher in hormone-treated versus control crickets (Fig. 4). When analyzed by ANCOVA with total lipid dpm as the covariate, phospholipid dpm was significantly higher in methoprene-treated versus control crickets over the three diets on the high-sucrose or low diets tested separately but not on the high diet tested alone. No diet × treatment interaction was observed. Thus, although hormone-treated crickets biosynthesize a

smaller amount of total lipid than control crickets, they allocate a significantly greater proportion of total lipid biosynthesis to phospholipid. The increased proportion of phospholipid in hormone-treated crickets was due to their proportionally greater biosynthesis of phosphatidyl choline (ANCOVA-adjusted means ± SEM pooled over the three diets: control: 576 ± 74 dpm [$n = 22$]; hormone: $1{,}088 \pm 65$ dpm [$n = 26$]; ANCOVA: $F_{1,41} = 21.1$, $P = 0.000$; no significant treatment × diet interaction, $P = 0.6$). Proportional biosynthesis of the other major phospholipid in *G. firmus*, phosphatidyl ethanolamine, did not differ between treatments (ANCOVA: $F_{1,41} = 0.164$, $P = 0.16$; no significant diet × treatment interaction, $P = 0.61$). Proportional incorporation of [^{14}C]-acetate into triglyceride was significantly higher in control versus hormone-treated females, with the results of ANCOVAs very similar to those of ANOVAs presented above for phospholipid.
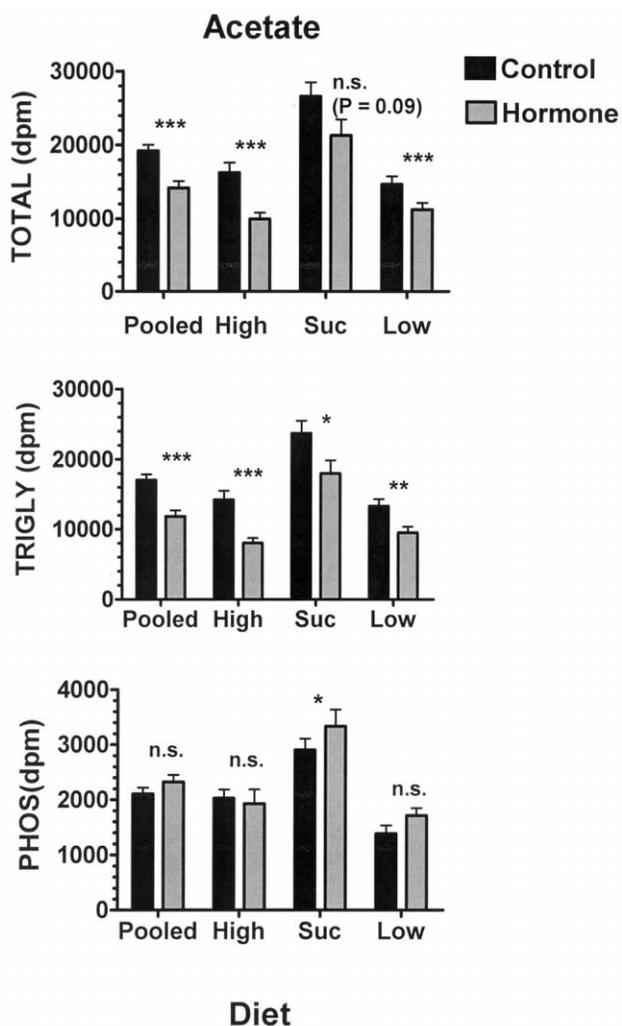
Figure 3. Radioactivity (mean DPM ± SEM) from [14C]-sodium acetate incorporated into various lipid classes in hormone-treated or control LW(f) *Gryllus firmus* fed various diets. *TOTAL* = total lipid; *TRIGLY* = triglyceride;   *PHOS* = phospholipid;   *High* = standard high-nutrient diet; *Suc* = high-sucrose diet; *Low* = low-nutrient diet. Results of ANOVA: *three asterisks* = $P < 0.005$; *two asterisks* = $P < 0.01$; *one asterisk* = $P < 0.05$; $n = 7–10$ for each unpooled mean.

Triglyceride and Phospholipid Biosynthesis from Palmitate

The effect of methoprene on lipid biosynthesis from the fatty acid [14C]-palmitate yielded important similarities as well as important differences compared with results of biosynthesis from [14C]-acetate presented above. Like the studies using acetate, a significantly lower absolute amount of [14C]-palmitate was incorporated into triglyceride in hormone-treated than was incorporated in control crickets on all diets and on the high and sucrose diets considered separately (Fig. 5, *middle*). By contrast, a substantially greater absolute amount of [14C]-palmitate was incorporated into phospholipid in hormone-

treated versus control crickets over all diets and on the high and sucrose diets tested separately. Differences between control and hormone-treated crickets with respect to incorporation of palmitate into triglyceride versus phospholipid counteracted each other, resulting in no difference in absolute incorporation of radiolabel from [14C]-palmitate into total lipid (Fig. 5, *top*). Finally, significant diet × treatment interactions were observed for palmitate incorporation into either triglyceride or phospholipid (ANOVA: $P < 0.005$). This was due to strong differences in incorporation between hormone and control crickets on the high and sucrose diets but not on the low diet (Fig. 5). Because incorporation into total lipid did not differ between treatments, absolute and relative biosynthesis of triglyceride versus phospholipid were similar (i.e., results of ANOVAs presented above are similar to results of ANCOVAs [data not shown]).

Lipid Oxidation

In general, measures of lipid oxidation using either [14C]-palmitate or [14C]-acetate gave similar results. Hormone-treated *G. firmus* oxidized a greater amount of either radiolabeled compound than did control crickets over all diets and on each diet tested separately (Fig. 6). However, for palmitate oxidation, a highly significant treatment × diet interaction was observed ($F_{2,39} = 6.58$, $P = 0.003$). This interaction was due to the proportionally greater oxidation of palmitate by hormone-treated versus control crickets on the high-sucrose diet (Fig. 6). No diet × treatment interaction was observed for [14C]-acetate oxidation (ANOVA, $P = 0.49$).
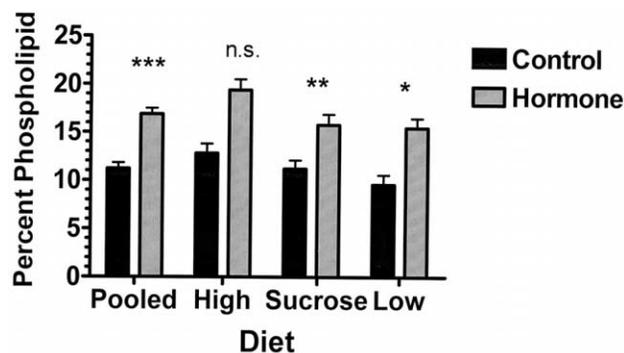

Figure 4. Percentage of total lipid radiolabel due to phospholipid in methoprene-treated and control *Gryllus firmus*. Percentages were based on samples of seven to 10 hormone-treated or control crickets within each diet treatment. [14C]-acetate was the radiolabel. Asterisks (denoting same values as those in Fig. 3) above histograms refer to results of ANCOVA comparing the proportional incorporation of radiolabel into phospholipid (radiolabeled phospholipid was the dependent variable, and radiolabeled total lipid was the covariate). In *G. firmus*, total lipid is defined as phospholipid plus triglyceride, which comprises greater than 90% of all lipid classes (see Zera and Larsen 2001; Zera and Zhao 2003b).
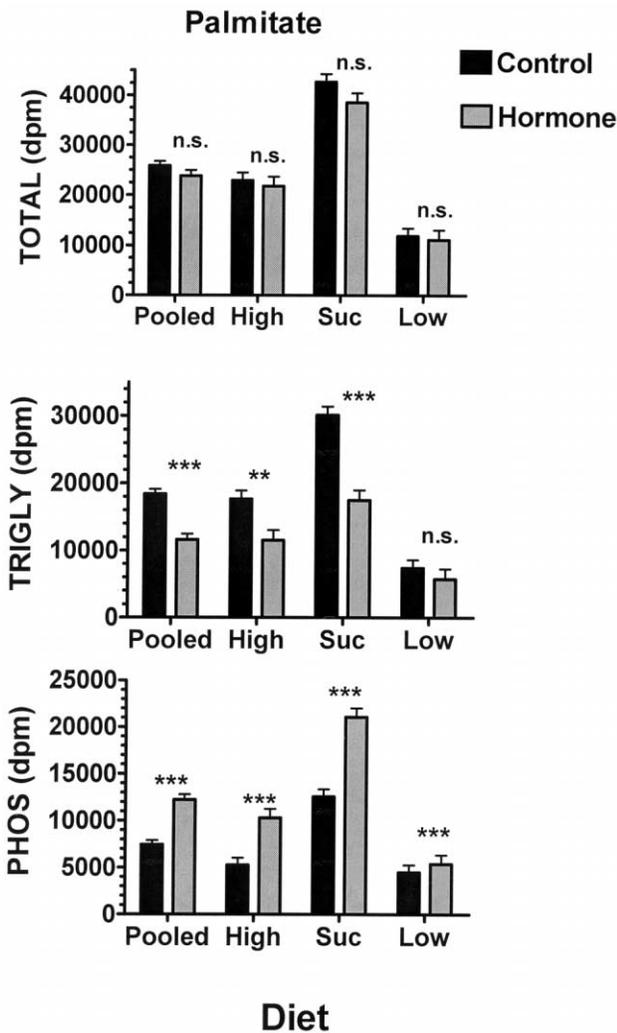
Figure 5. As in Figure 3, but using [^{14}C]-sodium palmitate as the radiolabel; $n = 6$–9 for each unpooled mean.

Correlations

In the first analysis of [^{14}C]-acetate incorporation, correlations between proportion of total radiolabel incorporated into each of two phospholipids, triglyceride, and CO_2 were tested. Radiolabel incorporated into CO_2 exhibited strong negative correlations with each of the three lipid components, while each of the three lipid components was positively correlated with the other two (Table 1, pt. A). When proportion of radiolabel incorporated into the three lipid classes was tested alone (i.e., radiolabel incorporated into CO_2 was not considered), strong negative correlations were observed between triglyceride and each of the two phospholipids, while the two phospholipids were positively correlated with each other (Table 1, pt. B). In the palmitate incorporation experiment, the two phospholipid classes were not separated. Thus, correlations between pro-

portional radiolabel incorporated into triglyceride versus phospholipid were uninformative (i.e., must equal -1.0; see "Methods") and were not tested. When triglyceride, phospholipid, and CO_2 were considered together, triglyceride exhibited strong negative correlations with either CO_2 ($r = -0.86$, $P < 0.01$) or phospholipid ($r = -0.54$, $P < 0.01$), while phospholipid and CO_2 were uncorrelated ($r = +0.08$, not significant; $n = 45$ in each test).

Discussion

Overview

The main finding of this study is that hormonal manipulation substantially altered lipid metabolism and the masses of organs of reproduction, flight, and intermediary metabolism in the flight-capable LW(f) morph of *Gryllus firmus*. As will be discussed below, traits expressed in LW(f) females after hormonal manipulation were remarkably similar to those observed in unmanipulated SW females of this species. These results strongly imply that key differences in lipid metabolism and reproduction between LW(f) and SW morphs are caused by modifications in endocrine regulation.
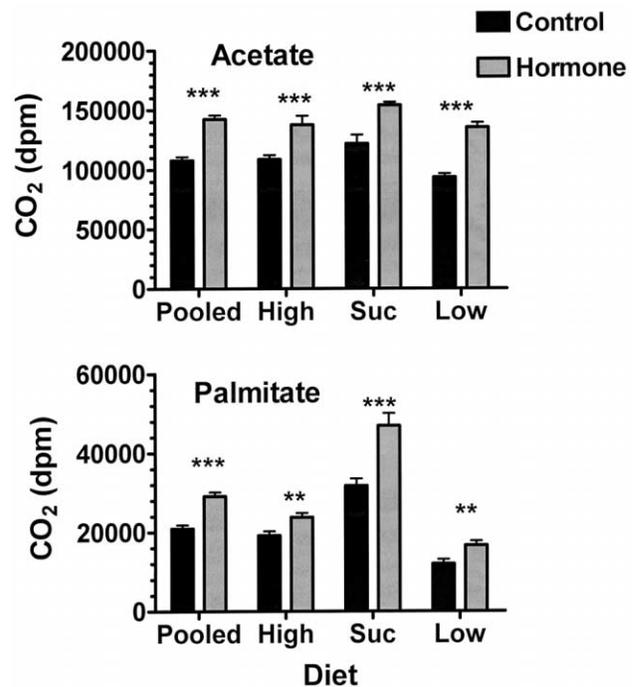

Figure 6. Radioactivity from [^{14}C]-acetate or [^{14}C]-palmitate incorporated into CO_2. Symbols are as in Figure 4; $n = 7$–10 (acetate) or 6–9 (palmitate) for each unpooled mean. The same individuals were used in these experiments as were used in experiments illustrated in Figures 3, 5.

Table 1: Spearman correlations between percentage of total radiolabel incorporated into various lipid components and CO_2 in methoprene-treated and control *Gryllus firmus*

First Variable	Other Variables		
	Triglyceride	Phos Choline	Phos Ethanolamine
A. Percentage radiolabel in lipid classes and CO_2:			
Triglyceride	...		
Phos choline	.547**	...	
Phos ethanolamine	.433*	.538*	...
CO_2	−.993**	−.606**	−.496*
B. Percentage radiolabel in lipid classes alone (i.e., excluding CO_2):			
Triglyceride	...		
Phos choline	−.827**	...	
Phos ethanolamine	−.845**	.477**	

Note. Radiolabel was [^{14}C]-acetate. See "Results" for correlations in experiment employing [^{14}C]-palmitate.

  * $P < 0.05$ from Table "Y" of Sokal and Rohlf (1969), taking into account multiple independent variables. Sample size was 45 individuals. See "Methods" for additional details.

  ** $P < 0.01$ from Table "Y" of Sokal and Rohlf (1969), taking into account multiple independent variables. Sample size was 45 individuals. See "Methods" for additional details.

Effect of Methoprene on Lipid Metabolism in LW(f) Gryllus firmus

Previous studies have shown that differences in lipid metabolism between flight-capable LW(f) and flightless SW *G. firmus* represent a key component of morph specialization for flight versus reproduction. During the first week of adulthood, the LW(f) morph prioritizes accumulation of triglyceride, the main flight fuel in *Gryllus* (Zera et al. 1999), at the expense of ovarian growth, while the opposite occurs in the SW morph (Zera and Larsen 2001). Increased accumulation of triglyceride in LW(f) females occurs via morph-specific differences in lipid metabolism and not by increased acquisition of lipid from the diet (Zera and Brink 2000). Relative to the SW morph, LW(f) females exhibit increased absolute biosynthesis of total lipid and triglyceride, increased proportional biosynthesis of triglyceride versus phospholipid, decreased oxidation of fatty acids, increased allocation of biosynthesized triglyceride to the soma versus eggs, and increased conversion of amino acids into triglyceride (Zhao and Zera 2001, 2002; Zera and Zhao 2003*a*, 2003*b*; A. J. Zera and Z. Zhao, unpublished data; Table 2). Reduced biosynthesis and increased oxidation of fatty acid in the SW morph likely provides energy for the massive biosynthesis of vitellogenin required for the enhanced ovarian growth in that morph (Zera and Zhao 2003*b*). A central issue addressed in this study is the physiological mechanisms that coordinate the expression of suites of biochemical, reproductive, and morphological traits, resulting in morphs that are specialized for flight versus reproduction.

Methoprene, a widely used JH analogue (Zera and Tiebel 1988; Nijhout 1994), applied to the flight-capable LW(f) morph caused this morph to express biochemical, morphological, and reproductive traits typically seen in the SW phenotype. In LW(f) females, methoprene dramatically reduced in vitro activities of lipogenic enzymes (Fig. 2) and total lipid and triglyceride lipid biosynthesis (Figs. 3, 5) but increased oxidation of fatty acids (Fig. 6) and absolute (Figs. 3, 5) and proportional biosynthesis of phospholipid (percentage of total lipid biosynthesis due to phospholipid; Fig. 4; Table 2). Collectively, methoprene diverted carbon away from triglyceride biosynthesis and into the production of phosphatidyl choline and energy (i.e., oxidation of fatty acids).

The magnitudes of biochemical differences between hormonally treated and control LW(f) females were quantitatively and strikingly similar to the magnitudes of differences in these traits between unmanipulated SW and LW(f) females reported in previous studies (Table 2). For example, on average, methoprene caused a 36% reduction in activities of lipogenic enzymes in LW(f) females relative to controls, similar to the 44% reduction in the activities of these enzymes in unmanipulated SW versus LW(f) females of the same age (Table 2). Likewise, methoprene-treated females exhibited percent reduction in triglyceride biosynthesis, percent increase in phospholipid biosynthesis, and percent increase in fatty acid oxidation that were roughly similar to those seen between unmanipulated SW versus LW(f) females (Table 2). Even the proportional conversion of fatty acids into phospholipid versus triglyceride was elevated to a similar degree in hormone-treated versus control LW(f) as in unmanipulated SW versus LW(f) females (Table 2). Finally, the magnitudes of change in ovarian and fat body mass between hormonally treated and control LW(f) females were similar to those between unmanipulated SW versus LW(f) females (Table 2).

Table 2: Percentage difference in aspects of lipid metabolism and ovarian and fat body mass between the genetically determined SW morph or the hormone-treated LW(f) morph (SW phenocopy) versus the LW(f) morph of *Gryllus firmus*

Trait Measured	Genetically Determined SW versus LW(f)	Hormonally Treated LW(f) (SW Phenocopy) versus LW(f) (Untreated Control)
Enzyme activity:		
FAS	−45% (H), −35% (S)	−26% (H), −36% (S)
ACL	−44% (H), −55% (S)	−22% (H), −54% (S)
G-6-PDH	−40% (H), −60% (S)	−51% (H), −43% (S)
NADP$^+$-IDH	−28% (H), −46% (S)	−36% (H), −43% (S)
AspAT	−27% (H), −45% (S)	−37% (H), −37% (S)
HOAD	−14% (H), −46% (S)	−18% (H), −20% (S)
Biosynthesis of lipid classes from acetate (Ace) or palmitate (Pal):		
Pal to triglyceride	−33% (P)	−31% (P)
Ace to triglyceride	−31% (P)	−31% (P)
Pal to phospholipid	+43% (P)	+64% (P)
Ace to phospholipid	+20% (P)	+10% (P)
Pal to phospholipid/total lipid	+50% (P)	+33% (P)
Oxidation of fatty acid (palmitate) or acetate to CO_2:		
Palmitate to CO_2	+52% (H), +44% (S), +75% (L)	+24% (H), +48% (S), +39% (L)
Acetate to CO_2	+33% (H), +24% (S), +18% (L)	+27% (H), +26% (S), +44% (L)
Organ wet masses:		
Ovaries	+266% (H), +151% (S), +394% (L)	+167% (H), +96% (S), +36% (L)
Fat bodies	−52% (H), −54% (S), −4% (L)	−41% (H), −24% (S), −9% (L)

Note. Comparisons between SW and LW(f) morphs are based on data from Zhao and Zera (2001, 2002) or Zera and Zhao (2003*a*, 2003*b*). Comparisons between hormonally treated LW(f) and control LW(f) are based on data from Figures 1–6. "H," "S," and "L" refer to data obtained on high, sucrose, and low diets, respectively, while "P" refers to data pooled across diets. See "Methods" for diet composition.

The effects of methoprene on lipid metabolism, ovarian growth, and fat body mass observed in this study were similar to effects of methoprene or JH manipulation reported in numerous earlier studies on a wide variety of non-wing-polymorphic insects. For example, total lipid or triglyceride increased in each of eight phylogenetically diverse insect species following allatectomy, a surgical procedure that removes the gland that produces JH (Steele 1985), and was reduced in the cricket *Gryllus assimilis* after application of methoprene (Zera et al. 1998). Allatectomy also increased lipid biosynthesis from radiolabeled acetate, incorporation of radiolabeled palmitate into triglycerides, and activities of lipogenic enzymes in various insect species (reviewed in Downer 1985). Application of a JH agonist caused a reduction in fatty acid biosynthesis in a lepidopteran (moth; Mulye and Gordon 1993). Finally, exogenous JH or JH agonists enhanced ovarian growth but reduced fat body mass in a wide variety of insects, while elimination of JH via allatectomy had the opposite effects (Zera and Denno 1997; Zera et al. 1998).

In this study, changes in the various aspects of lipid metabolism and ovarian and fat body mass associated with methoprene application were within the range of differences typically seen between unmanipulated morphs in *G. firmus* (Table 2). Moreover, in no case did we observe an effect of methoprene in *G. firmus* that was inconsistent with effects of either methoprene or JH itself reported in earlier studies of other species. This suggests that methoprene-induced responses observed in this study were physiological rather than pharmacological. In other words, experimentally produced alterations in lipid metabolism observed in this study likely mimic differences in lipid metabolism between unmanipulated morphs that arise from naturally occurring variation in in vivo hormonal regulation.

The main importance of this study is that it provides a mechanism to account for the expression of alternate suites of ge-

netically determined metabolic, morphological, and reproductive traits in LW(f) and SW morphs of *G. firmus*. Genetically specified variation in endocrine regulation appears to coordinate expression of elevated triglyceride biosynthesis, reduced phospholipid biosynthesis, reduced fatty acid oxidation, elevated activities of numerous lipogenic enzymes, reduced ovarian growth, and so on, in the LW(f) and the complement of these traits in the SW morph (Zhao and Zera 2002; Zera and Zhao 2003*a*, 2003*b*; Table 2).

Endocrine Regulation of Intermediary Metabolism by Juvenile Hormones and Other Hormones

Hormones coordinate the expression of numerous genes that encode enzymes of intermediary metabolism (Granner and Pilkis 1990; Sul and Wang 1998; Zhou et al. 1998). Ferea et al. (1999) have provided evidence suggesting that microevolutionary alteration of the expression of hundreds of genes encoding metabolic enzymes can occur via selection on a few variable regulators. Thus, the existence of genetically variable endocrine regulators and/or regulators that coordinate morph-dependent expression of many aspects of lipid metabolism in *G. firmus* is not unexpected. Nevertheless, to our knowledge, this is the first evidence for endocrine regulation of a life-history trade-off operating via hormonal effects on specific metabolic pathways and enzymes of intermediary metabolism.

Although this study provides strong evidence that the differential expression of aspects of lipid metabolism in LW(f) and SW morphs of *G. firmus* are hormonally regulated, the specific hormonal mechanisms involved are unclear. Morph-specific differences in lipid metabolism and ovarian growth might be due to direct pleiotropic effects of variation in JH regulation (e.g., morph-specific differences in the JH titer or receptor number/affinity). Alternatively, JH might regulate an endocrine cascade and might not directly control individual aspects of metabolism. Finally, JH itself might not be involved in the in vivo regulation of morph-specific differences in lipid metabolism; the effects of methoprene observed in this study might have resulted from the induction of as yet unidentified hormonal regulators. Exogenous JH and JH analogues can strongly alter the in vivo titers of other hormones such as ecdysteroids or neuropeptides (Smith and Nijhout 1981; Zera and Tiebel 1988; Stay and Woodhead 1993).

The regulation of metabolism in insects by JH or other hormones is currently not well understood (reviewed in Downer 1985; Steele 1985). For example, although strong effects of JH or methoprene on many aspects of metabolism have been reported for decades, in no insect is it known whether JH is directly or indirectly involved in such responses or what the mechanisms involved are. In addition, although evidence for non-JH hormones that positively or negatively affect lipid or carbohydrate metabolism in insects has been reported for decades (Downer 1985; Steele 1985), with the notable exception

of adipokinetic hormone (Orchard 1987; Gade 1990), little is known about the identity of these hormones or their mode of action.

JH titer differences between morphs of *G. firmus* are also equivocal with respect to providing insights into the endocrine mechanisms that control morph-specific differences in lipid metabolism in this species. A long-standing notion regarding the endocrine regulation of wing polymorphism is that a JH titer above some threshold regulates the expression of traits that define the flightless morph, while a JH titer below that threshold regulates the expression of the alternate set of traits that specify the flight-capable morph (Nijhout 1994; Zera and Denno 1997; Zera and Harshman 2001). However, the first direct measurement of the JH titer in a wing-polymorphic insect (*G. firmus*; Zera and Cisper 2001) documented that hormone titer differences between morphs are more complex than previously suspected. In brief, the JH titer in the SW morph is temporally constant, while the titer rises 10–100-fold during the late photophase in the LW(f) morph. Temporal variation in the JH titer in the LW(f) morph results in titers that are substantially higher in the LW(f) versus SW morph at the end of the photophase and beginning of the scotophase but are lower in the LW(f) morph or are equivalent in both morphs during other times of the day. In contrast to the complex pattern of JH titer variation between the morphs, experimental manipulation of the JH titer in LW(f) *G. firmus* and other crickets has led to a straightforward and consistent result: long-duration (d) elevation of the JH titer in LW(f) females causes them to express traits normally seen in the SW morph such as enhanced growth of the ovaries, reduction of flight muscles, and reduction of lipid reserves (Zera et al. 1998; Zera and Cisper 2001; and references therein). Zera and Cisper (2001) proposed several endocrine mechanisms that can reconcile the paradoxical results obtained from direct quantification of the in vivo JH titer and experimental manipulation of in vivo JH levels. For example, long-duration elevation in the JH titer (i.e., greater than 12 h) may be required to express traits observed in SW females. The short-duration (<12 h) spike in the JH titer in the LW(f) morph may positively regulate specific behaviors associated with nocturnal flight without causing the expression of traits detrimental to flight (e.g., flight muscle histolysis, extensive ovarian growth). In this study, we used multiple applications of the long-acting JH agonist methoprene to produce a long-duration elevation of the effective JH titer in the LW(f) morph over a period of days. The expression of aspects of lipid metabolism and ovarian growth in these LW(f) females that are typically seen in the SW morph is consistent with the "JH-duration" hypothesis of Zera and Cisper (2001) mentioned above. We emphasize that this hypothesis is speculative and that additional data are required to adequately support or refute it.

Morph-dependent diurnal changes in the JH titer in *G. firmus* raises the possibility that other traits may differ between morphs

in a time-dependent manner. However, this is not the case for reproductive or biochemical traits measured to date. For example, ovarian mass was higher in SW versus LW(f) females to a similar degree throughout the photophase or scotophase (Zera and Cisper 2001; A. J. Zera and Z. Zhao, unpublished data). Similarly, differences between morphs in specific activities of enzymes of lipid metabolism, rates of lipid biosynthesis, and ecdysteroid titers are of roughly the same magnitude early or late in the photophase (A. J. Zera and Z. Zhao, unpublished data). Therefore, accurate measurement of relative differences between morphs in aspects of lipid metabolism and reproduction requires only that traits be measured at one and the same time during the day.

Implications for Endocrine-Biochemical Basis of Life-History Evolution

During the past decade there has been an increasing consensus that variation in hormonal regulation plays a cardinal role in life-history variation and trade-offs (Ketterson and Nolan 1999; Sinervo 1999; Zera and Harshman 2001). Yet details of the mechanisms involved are poorly understood (Zera and Harshman 2001). This is especially true with respect to aspects of life-history variation and trade-offs that result from alteration in the hormonal control of intermediary metabolism. Indeed, to our knowledge this study represents the first detailed investigation of this topic.

There appears to be several reasons why the hormonal control of pathways of intermediary metabolism that contribute to life-history trade-offs has been ignored. First, until very recently, intermediary metabolism itself has been treated as a black box in life-history studies (Zhao and Zera 2001). Except for a few rare cases (Zhao and Zera 2001, 2002; O'Brien et al. 2002; Zera and Zhao 2003a, 2003b), little is known about variation or covariation in specific enzymes or pathways of intermediary metabolism that underlie variation in life-history traits or trade-offs. Such information is a prerequisite for investigating the mechanisms by which endocrine regulation underlies a life-history trade-off by virtue of its modulatory effects on intermediary metabolism. Second, the influence of metabolism on life-history evolution has almost exclusively been studied from an energetics perspective (Zera and Harshman 2001). That is, the main focus has been quantitative or qualitative assessment of calories allocated to various organismal processes that underlie various life-history traits and trade-offs. The regulatory mechanisms that control variation in internal resource allocation have rarely been discussed, let alone investigated. By contrast, this study clearly shows the importance of alterations in the hormonal regulation of intermediary metabolism as a potentially cardinal factor in the trade-off between nutrients devoted to the soma (i.e., flight capability) versus reproduction.

Literature Cited

Christie W.W. 1982. Lipid Analysis. Pergamon, Oxford.

Dhadialla T.S. and G.R. Wyatt. 1981. Competence for juvenile-hormone-stimulated vitellogenin synthesis in the fat body of female and male *Locusta migratoria*. Pp. 257–262 in G.E. Pratt and G.T. Brooks, eds. Juvenile Hormone Biochemistry. Elsevier, Amsterdam.

Djawdan M., T.T. Sugiyama, L.K. Schlaeger, T.J. Bradley, and M.R. Rose. 1996. Metabolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. Physiol Zool 69:1176–1195.

Doughty P. and R. Shine. 1998. Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). Ecology 79:1073–1083.

Downer R.G.H. 1985. Lipid metabolism. Pp. 77–113 in G.A. Kerkut and L.I. Gilbert, eds. Comprehensive Insect Biochemistry, Physiology and Pharmacology. Vol. 10. Pergamon, Oxford.

Ferea T.L., D. Botstein, P.O. Brown, and R.F. Rosenzweig. 1999. Systematic changes in gene expression patterns following adaptive evolution in yeast. Proc Natl Acad Sci USA 96:9721–9726.

Fisher R.A. 1930. The Genetical Theory of Natural Selection. Dover, New York.

Gade G. 1990. The adipokinetic hormone/red pigment concentrating hormone peptide family: structures, interrelationships and functions. J Insect Physiol 36:1–12.

Geer B.W. and C.C. Laurie-Ahlberg. 1984. Genetic variation in the dietary sucrose modulation of enzyme activities in *Drosophila melanogaster*. Genet Res 43:307–321.

Granner D. and S. Pilkis. 1990. The genes of hepatic glucose metabolism. J Biol Chem 265:10173–10176.

Harshman L.G. and J.L. Schmidt. 1998. Evolution of starvation resistance in *Drosophila melanogaster*: aspects of metabolism and counter-impact selection. Evolution 52:1679–1685.

Ketterson E.D. and V. Nolan. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. Am Nat 154(suppl.):S4–S25.

Mulye H. and R. Gordon. 1993. Effects of fenoxycarb, a juvenile hormone analog, on lipid metabolism of the eastern spruce budworm. J Insect Physiol 39:721–727.

Nijhout H.F. 1994. Insect Hormones. Princeton University Press, Princeton, N.J.

Norris D.O. 1997. Vertebrate Endocrinology. Academic Press, San Diego, Calif.

O'Brien D.M., M.L. Marilyn, and C. Boggs. 2002. Renewable and nonrenewable resources: amino acid turnover and al-

location to reproduction. Proc Natl Acad Sci USA 99:4413–4418.

Orchard I. 1987. Adipokinetic hormones: an update. J Insect Physiol 33:451–463.

Ricklefs R.E. 1996. Avian energetics, ecology and evolution. Pp. 1–30 in C. Cary, ed. Avian Energetics. Chapman, New York.

Riddiford L.M. 1985. Hormone action at the cellular level. Pp. 37–84 in G.A. Kerkut and L.I. Gilbert, eds. Comprehensive Insect Biochemistry, Physiology and Pharmacology. Vol. 8. Pergamon, Oxford.

Rose M.R. and T.J. Bradley. 1998. Evolutionary physiology of the cost of reproduction. Oikos 83:443–451.

Service P.M. 1987. Physiological mechanisms of increased stress resistance in Drosophila melanogaster selected for postponed senescence. Physiol Zool 60:321–326.

Sinervo B. 1999. Mechanistic analysis of natural selection and a refinement of Lack's and Williams's principles. Am Nat 154(Suppl.):S26–S42.

Smith W. and H.F. Nijhout. 1981. Effects of a juvenile hormone analogue on duration of the fifth instar in the milkweed bug Oncopeltus fasciatus. J Insect Physiol 27:169–173.

Sokal R.R. and F.J. Rohlf. 1969. Statistical Tables. Freeman, San Francisco.

Stay B. and A.P. Woodhead. 1993. Neuropeptide regulators of insect corpora allata. Am Zool 33:357–364.

Steele J.H. 1985. Control of metabolic processes. Pp. 99–146 in G.A. Kerkut and L.I. Gilbert, eds. Comprehensive Insect Biochemistry, Physiology and Pharmacology. Vol. 10. Pergamon, Oxford.

Sul H.S. and D. Wang. 1998. Nutritional and hormonal regulation in fat synthesis: studies of fatty acid synthase and mitochondrial glycerol-3-phosphate acyltransferase gene transcription. Annu Rev Nutr 18:331–351.

Townsend C.R. and P. Calow. 1981. Physiological Ecology: An Evolutionary Approach to Resource Use. Blackwell Scientific, Oxford.

Williams T.D. and F. Vezina. 2001. Reproductive energy expenditure, intraspecific variation, and fitness. Curr Ornithol 16:355–405.

Wyatt G.R. and K.G. Davey. 1996. Cellular and molecular actions of juvenile hormone. II. Roles of juvenile hormone in adult insects. Adv Insect Physiol 26:1–155.

Zera A.J. and T. Brink. 2000. Nutrient absorption and utilization by wing and flight muscle morphs of the cricket Gryllus firmus: implications for the trade-off between flight capability and early reproduction. J Insect Physiol 46:1207–1218.

Zera A.J. and G. Cisper. 2001. Genetic and diurnal variation in the juvenile hormone titer in a wing-polymorphic cricket: implications for the evolution of life histories and dispersal. Physiol Biochem Zool 74:293–306.

Zera A.J. and R.F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annu Rev Entomol 42:207–231.

Zera A.J. and L.G. Harshman. 2001. Physiology of life history trade-offs in animals. Annu Rev Ecol Syst 32:95–126.

Zera A.J. and Y. Huang. 1999. Evolutionary endocrinology of juvenile hormone esterase: functional relationship with wing polymorphism in the cricket, Gryllus firmus. Evolution 53:837–847.

Zera A.J. and A. Larsen. 2001. The metabolic basis of life history variation: genetic and phenotypic differences in lipid reserves among life history morphs of the wing-polymorphic cricket, Gryllus firmus. J Insect Physiol 47:1147–1160.

Zera A.J., J. Potts, and K. Kobus. 1998. The physiology of life-history trade-offs: experimental analysis of a hormonally induced life-history trade-off in Gryllus assimilis. Am Nat 152:8–23.

Zera A.J., J. Sall, and K. Grudzinski. 1997. Flight-muscle polymorphism in the cricket Gryllus firmus: muscle characteristics and their influence on the evolution of flightlessness. Physiol Zool 70:519–529.

Zera A.J., J. Sall, and K. Otto. 1999. Biochemical aspects of flight and flightlessness in Gryllus: flight fuels, enzyme activities, and electrophoretic profiles of flight muscles from flight-capable and flightless morphs. J Insect Physiol 45:275–285.

Zera A.J. and K.C. Tiebel. 1988. Brachypterizing effect of group rearing, juvenile hormone-III, and methoprene on wing-length development in the wing-dimorphic cricket, Gryllus rubens. J Insect Physiol 34:489–498.

Zera A.J. and Z. Zhao. 2003a. Life-history evolution and the microevolution of intermediary metabolism: activities of lipid-metabolizing enzymes in life history morphs of a wing-dimorphic cricket. Evolution 57:586–596.

———. 2003b. Morph-dependent fatty-acid oxidation in a wing-polymorphic cricket: implications for the trade-off between dispersal and reproduction. J Insect Physiol 49:933–943.

Zhao Z. and A.J. Zera. 2001. Enzymological and radiotracer studies of lipid metabolism in the flight-capable and flightless morphs of the wing-polymorphic cricket, Gryllus firmus. J Insect Physiol 47:1337–1347.

———. 2002. Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket. Proc Natl Acad Sci USA 99:16829–16834.

Zhou Y., M. Shimabukuro, K. Koyama, Y. Lee, and M. Wang. 1998. Induction by leptin of uncoupling protein-2 and enzymes of fatty acid oxidation. Proc Natl Acad Sci USA 94:6386–6390.