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Intermediary Metabolism and Life History Trade-offs: Lipid Metabolism in Lines of the Wing-polymorphic Cricket, *Gryllus firmus*, Selected for Flight Capability *vs.* Early Age Reproduction[1]

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Synopsis: The extent to which modifications in intermediary metabolism contribute to life history variation and trade-offs is an important but poorly understood aspect of life history evolution. Artificial selection was used to produce replicate genetic stocks of the wing-polymorphic cricket, *Gryllus firmus*, that were nearly pure-breeding for either the flight-capable (LW[f]) morph, which delays ovarian growth, or the flightless (SW) morph, which exhibits enhanced early-age fecundity. LW(f) lines accumulated substantially more triglyceride, the main flight fuel in *Gryllus*, compared with SW-selected lines, and enhanced accumulation of triglyceride was strongly associated with reduced ovarian growth. Increased triglyceride accumulation in LW(f) lines resulted from elevated *de novo* biosynthesis of fatty acid and two morph-specific trade-offs: (1) greater proportional utilization of fatty acid for glyceride biosynthesis *vs.* oxidation, and (2) a greater diversion of fatty acids into triglyceride *vs.* phospholipid biosynthesis. Even though SW lines produced less total lipid and triglyceride, they produced more phospholipid (important in egg development) than did LW(f) lines. Differences between LW(f) and SW morphs in lipid biosynthesis resulted from substantial alterations in the activities of all studied lipogenic enzymes, a result that is consistent with expectations of Metabolic Control Theory. Finally, application of a juvenile hormone analogue to LW(f) females produced a striking SW phenocopy with respect to all aspects of lipid metabolism studied. Global alterations of lipid metabolism, most likely produced by alterations in endocrine regulation, underlie morph specializations for flight *vs.* early-age fecundity in *G. firmus*. Modification of the endocrine control of intermediary metabolism is likely to be an important mechanism by which intermediary metabolism evolves and contributes to life history evolution.

Introduction

The functional causes of life-history evolution have been a central focus of life-history research for over six decades (Fisher, 1930; Pianka, 1981; Townsend and Calow, 1981; Ricklefs, 1996; Rose and Bradley, 1998; Zera *et al.*, 1998; Ketterson and Nolan, 1999; Sinervo *et al.*, 2000; Zera and Harshman, 2001; Ricklefs and Wikelski, 2002; Zhao and Zera, 2002). At issue are the physiological, biochemical, and molecular processes that have been altered by natural selection to produce modified life-history traits (*e.g.,* enhanced early-age reproduction), and trade-offs between traits (*e.g.,* enhanced early-age reproduction coupled with decreased longevity). A number of studies have identified physiological (*e.g.,* energetic and endocrine) correlates of life-history variation and trade-offs within species (Service, 1987; Djawdan *et al.*, 1996; Rose and Bradley, 1998; Sinervo *et al.*, 2000; Ketterson and Nolan, 1999; Zera and Cisper, 2001; Harshman and Hoffmann, 2000; Zera and Larsen, 2001; Zera and Harshman, 2001). More recently, other studies have begun to focus on the molecular causes of life history evolution (Stearns and Magwene, 2003). However, the biochemical-metabolic underpinnings of life history variation and trade-offs remain understudied aspects of life history evolution (Zhao and Zera, 2002; Zera and Zhao, 2003*b*).

A priori, evolutionary modification of intermediary metabolism is expected to be an important factor in life-history evolution. For example, the evolution of increased egg yolk protein biosynthesis is likely to be a key component of the evolution of increased early age fecundity. Similarly, increased longevity or resistance to stressful conditions (*e.g.,* tolerance to desiccation or starvation) are life history traits that are typically associated with enhanced accumulation of energy reserves, and must have evolved via modifications of carbohydrate and lipid metabolism (Zera and Harshman, 2001). Although modification of intermediary metabolism likely plays a key role in life history evolution, only limited information is available on any aspect of this topic (*e.g.,* specific metabolic pathways, enzymes, or regulatory controls that have been altered in genotypes, populations or species to produce differences in life histories). This is especially the case for genetically-based alterations in intermediary metabolism that underlie life history variation and trade-offs. This paucity of information constitutes a major roadblock to attaining a deep understanding of the mechanisms of life-history evolution (Zera and Harshman, 2001; Zera and Zhao, 2003*b*).

Artifical selection is a powerful tool to investigate the mechanisms of evolution (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Gibbs, 1999; Harshman and Hoffmann, 2000). Recently, a number of studies have begun to use artificial selection to investigate modifications of intermediary metabolism that underlie various aspects of life history evolution (Harshman and Schmidt, 1998; Harshman *et al.*, 1999; Harshman and Hoffmann, 2000; Zera and Larsen, 2001; Zhao and Zera, 2002;

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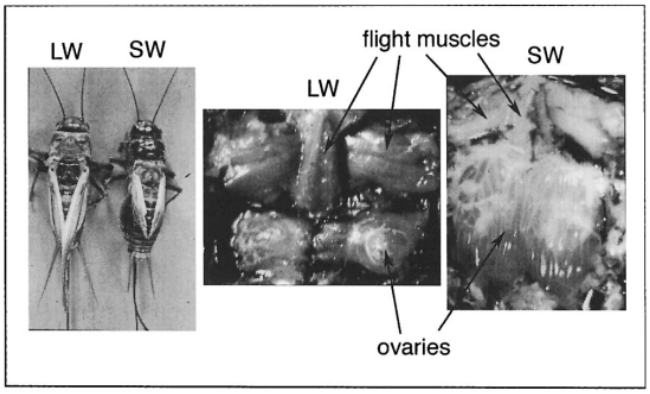

Figure. 1. Flight-capable (LW[f]; denoted as "LW" in this figure) and flightless (SW) female morphs of *Gryllus firmus* of the same age (day 5 of adulthood). In the left panel, the fore wings have been removed to show variation in the hind wings. The middle and right panels illustrate dissections of morphs showing much larger, functional flight muscles, but much smaller ovaries, in the flight-capable female, and substantially-underdeveloped flight muscles but much larger ovaries in the flightless female. See Figure 1 (Zera, 2004) for a color illustration of this figure.

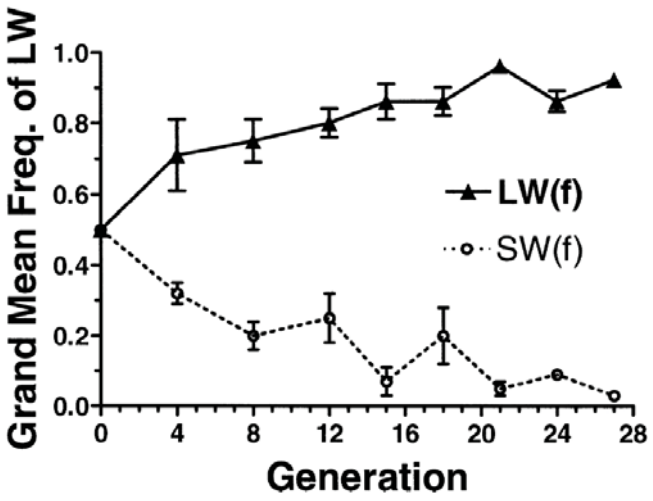

Figure 2. Response to artificial selection on wing morph (LW[f] or SW) in *G. firmus*. Solid line is the grand mean frequency of LW(f) females (mean of the three LW[f]-selected line means ± SEM), while the broken line is the grand mean frequency of the SW females (mean of three line means) as a function of generation of selection. Approximately 120 males and 120 females of the same morph type were used as breeders for each generation in each line. Females used in biochemical experiments were raised under slightly lower density which increased the frequency of the LW(f) morph in LW(f)-selected lines (to .90%), while keeping the freq. of the SW morph at .90% in SW-selected lines.

Zera and Zhao, 2003*a*, *b*). This review primarily focuses on results obtained in my laboratory during the past five years on variation in aspects of lipid metabolism between genetic stocks of the wing-polymorphic cricket, *Gryllus firmus*, artificially selected to produce morphs adapted for the flight at the expense of reproduction, and vice versa. The ultimate goal of these studies has been to identify genetically-based alterations in lipid metabolism that contribute to the trade-off between flight and reproduction. To put these studies in context I first provide background information on wing polymorphism, and the relationship between lipid physiology and life history trade-offs.

Background on Wing Polymorphism in *Gryllus firmus*

Wing polymorphism consists of morphs (discontinuous phenotypes) within a species that are adapted for flight at the expense of reproduction and vice versa (Fig. 1). The polymorphism is common in many insect groups, most notably the Hemiptera/Homoptera (waterstriders, planthoppers and aphids), Coleoptera (beetles) and, Orthoptera (crickets and grasshoppers) (Johnson, 1969; Harrison, 1980; Roff, 1986; Dingle, 1996; Zera and Denno, 1997). Natural populations of the cricket, *G. firmus*, the focus of this review, contain flight-capable and flightless morphs (Veazy *et al.*, 1976). The flight-capable morph (denoted LW[f]; [Zera *et al.*, 1997]; Fig. 1) has fully-developed wings, large (functional) flight muscles, and a large reserve of triglyceride, the main flight fuel in *Gryllus* (Zera *et al.*, 1999; Zera and Larsen, 2001). The alternate, flightless (SW) morph has underdeveloped, non-functional wings and flight muscles, and reduced whole-body triglyceride. By the end of the first week of adulthood, the SW morph has substantially elevated fecundity (100–400%) relative to the LW(f) morph (Fig. 1; Zera and Denno, 1997; Zera and Harshman, 2001; see below). Reproductive differences between morphs occur in the absence of flight, and thus reflect the negative impact of flight *capability* (production and maintenance of the flight apparatus), rather than flight itself, on egg production. Wing polymorphism is the most dramatic example of the trade-off between flight capability and reproduction, a life-history trade-off of prime important in insects in general (Johnson, 1969; Harrison, 1980; Roff, 1986; Dingle, 1996; Zera and Denno, 1997).

As is the case for most other wing-polymorphic insects, morph expression in *G. firmus* is influenced by both genetic and environmental factors (*i.e.,* it is a genetic polymorphism and an environmental polyphenism). Wing morph expression is under polygenic control (heritability = ~0.60–0.7; Roff, 1990), and nearly pure-breeding lines for the LW(f) or SW morphs have been produced in several laboratories by artificial selection (Roff, 1990; Zera and Cisper, 2001; Fig. 2). Environmental factors such as density, photoperiod, temperature, and food quality, also strongly affect morph expression in *G. firmus* and in other insects (Denno *et al.*, 1985; Zera and Tiebel, 1988; Zera and Denno, 1997).

Extensive ecological studies over many decades, indicate that wing polymorphism is maintained by environmental heterogeneity (Vepsalainen, 1978; Denno *et al.*, 1980, 1991; Roff, 1994; Dingle, 1996; Zera and Denno, 1997). Within a patch, the more-fecund flightless morph has a substan-

tial fitness advantage over the flight-capable morph. However, the flight-capable morph has the ability to track resources and mates between patches, as well as to escape deteriorating patches (Denno *et al.*, 1980; Langellato and Denno, 2001).

The physiological causes of morph specialization for flight capability *vs.* reproduction have been extensively studied in *G. firmus* and several congeners (Zera and Denno, 1997; Zera and Harshman, 2001). These studies provide important background context for biochemical investigations of morph specialization. Feeding studies in three species of *Gryllus* have documented that flight-capable and flightless morphs consume and assimilate equivalent or nearly equivalent amounts of nutrients (Mole and Zera, 1993; Zera *et al.*, 1998; Zera and Brink, 2000; A. J. Zera, unpublished data; discussed in more detail below). Thus, differences between the morphs in energy allocated to the flight capability *vs.* reproduction must be derived almost exclusively from morph-specific differences in internal nutrient allocation (*i.e.,* an internal trade-off) rather than from morph-specific differences in acquisition of nutrients from the diet. The LW(f) morph allocates a greater amount of its energy budget to the growth and maintenance of the large flight muscles and production of large quantities of triglyceride flight fuel (see below), which appear to constrain ovarian growth. At present, it is unclear whether reduced ovarian growth is due to nutrients or space within the LW(f) morph that are insufficient to accommodate large ovaries, in addition to the large thoracic muscles and large lipid reserves necessary for flight (Zera and Harshman, 2001). Alternatively, the endocrine environment necessary to accumulate large triglyceride reserves and to maintain the large flight muscles may have inherent negative effects on ovarian growth independent of nutrient availability (Zera and Cisper, 2001; Zera *et al.*, 1998; see below).

Lipids and Life Histories in Insects: Correlations Between Lipid Reserves and Life History Traits

Lipid is a heterogeneous class of molecules which plays a variety of important biological roles (Downer, 1985; Beenakkers *et al.*, 1985). In insects, triglyceride and phospholipid are the two most abundant lipid classes and together comprise more than 90% of total lipid (Beenakkers, 1985; Grapes *et al.*, 1989). Triglyceride is the most abundant energy storage molecule, while phospholipid, the second most abundant lipid class, is the primary component of biological membranes. In life history studies, lipid content has most often been investigated in the context of somatic (non-reproductive) energy stores (Zera and Harshman, 2001). However, eggs also contain a high content of both triglyceride and phospholipid and lipid biosynthesis increases during egg production (Beenakkers *et al.*, 1985; Grapes *et al.*, 1989; Lipsitz and McFairlane, 1970). Total lipid can be easily extracted by organic solvents and quantified, even in small insects like *Drosophila*. Thus, many life history studies in insects (most studies of *D. melanogaster*; Zera and Harshman, 2001) have measured whole-body, total lipid (or "neutral" lipid = *primarily* non-structural lipid energy reserves) to estimate calories devoted to somatic energy reserves. This method only gives a crude estimate of somatic lipid energy stores because a significant proportion of total extracted lipid may be due to structural lipid (*e.g.,* phospholipid). Furthermore, whole-body total or "neutral" lipid may contain a significant amount of non-somatic lipid (*e.g.,* from the ovaries), and, depending upon the specific extraction procedure, a variety of carbohydrates and amino acids (Christie, 1982). Other studies have documented differences between life-history phenotypes with respect to specific lipid classes, such as triglyceride (Nwanze *et al.*, 1976; Gunn and Gatehouse, 1993; Zera and Larsen, 2001), sometimes measured in specific organs (see below), which provide a more accurate picture of the functional relationship between various lipids and life history traits.

Several important associations have been identified between total lipid, or triglyceride, and specific life-history traits in insects. For example, total lipid/triglyceride is typically more abundant in individuals adapted to live longer, to withstand starvation or stress, or in preparation for energy demanding activities such as diapause, or flight (reviewed in Downer, 1985; Dingle, 1996; Zera and Harshman, 2001). Increased lipid/ triglyceride reserves for these demanding activities can accumulate during the juvenile stage (*e.g.,* Nwanze *et al.*, 1976; Gunn and Gatehouse, 1993; Chippendale *et al.*, 1996), and may be associated with slower juvenile growth rate (Chippendale *et al.*, 1996). Alternatively, these reserves may accumulate during adulthood and are often associated with reduced early-age fecundity (Djawdan *et al.*, 1998; Zera and Larsen, 2001; Zera and Harshman, 2001). The most extensive study of lipid classes in the context of a life history trade-off has been reported by Zera and Larsen (2001), and is discussed in detail below.

Consistent correlations between lipid levels and specific life history traits, discussed above for insects, also occur in animals in general (Zera and Harshman, 2001), and strongly suggest that alterations in lipid metabolism are a common aspect of life history evolution. However, until a few years ago, information on specific modifications of lipid metabolism that underlie life history adaptations has been restricted to a few enzymological characterizations of selected lines (Harshman and Schmidt, 1998; Harshman *et al.*, 1999). Essentially no information has been available on variation in *in vivo* processes such as rates of lipid biosynthesis or oxidation that underlie life history adaptations and trade-offs. Furthermore, interactions among pathways are an important aspect of intermediary metabolism (*e.g.,* pathways of protein and lipid metabolism are linked via the ability of amino acids to be converted into either protein or fatty-acid; Downer, 1985). Yet no information is available on the extent to which modification of pathway interactions contributes to life history adaptation. Finally, flux through pathways of intermediary metabolism is tightly regulated by hormones (Granner and Pilkis, 1996; Sul and Wang, 1998). Yet we are almost completely ignorant of alterations in the endocrine control of

TABLE 1. *Morph-specific trade-off in the proportional biosynthesis of triglyceride vs. phospholipid in* G. firmus.

Line type	Block 1	Block 2	Block 3	Results of paired *t*-test of line means
		Radiolabel: ^{14}C-Acetate		
LW(f)	84.2 ± 0.8 (26)**[a,b]	88.3 ± 0.5 (22)**	86.3 ± 0.8 (24)**	$t_{(2)} = 7.38$**[c]
SW	76.0 ± 1.3 (23)	82.4 ± 1.9 (24)	79.1 ± 1.1 (24)	$P < 0.02$
		Radiolabel: ^{14}C-Palmitic acid (fatty acid)		
LW(f)	69.7 ± 1.7 (20)**	64.5 ± 2.4 (21)**	67.7 ± 3.3 (22)**	$t_{(2)} = 4.90$
SW	45.3 ± 2.0 (20)	46.9 ± 2.2 (23)	51.6 ± 2.4 (23)	$P < 0.04$

[a] Values are mean percentage (±SEM) of radiolabelled acetate or palmitate converted into (triglyceride/(phospholipid + triglyceride) for the 3 pairs of LW(f) and SW-selected lines. Note the consistently greater proportional biosynthesis of triglyceride vs. phospholipid in LW(f) *vs.* SW lines (Data from Zhao and Zera, 2002).

[b] Asterisks represent results of unpaired *t*-tests comparing LW(f) and SW morphs within a block.

[c] Paired *t*-tests comparing means of selected lines across blocks. *t*-tests within and across blocks were performed on ANCOVA-adjusted numerators of the percentages with denominators as the covariate (see Zhao and Zera, 2002).

metabolism that have given rise to life history adaptations. In the past few years, detailed biochemical studies of lipid metabolism have been undertaken in artificially-selected lines of the wing-polymorphic cricket, *Gryllus firmus*, that have investigated each of the topics mentioned above. This work is the focus of the rest of the present review.

DIFFERENCES IN LIPID METABOLISM BETWEEN LIFE-HISTORY MORPHS OF *G. FIRMUS*

Artificial selection

To investigate the biochemical-genetic basis of life history variation and trade-offs, lines of *G. firmus* that produce a high frequency of either the flight-capable (LW[f]) morph or the flightless (SW) morph were obtained by artificial selection (Fig. 2). Details of artificial selection can be found in Zera and Larson (2001) and Zera and Cisper (2001). Briefly, selection was conducted in three temporally-separated blocks. Each block contained a line selected for the LW(f) morph, a line selected for the SW morph, and a control line. Control lines are not discussed here. All lines were initiated within a few weeks from a single base population, which, in turn, had been founded from *G. firmus* collected in Gainesville, Florida (Zera and Cisper, 2001). During selection, crickets were raised at 28°C, under a 16 light : 8 dark cycle, and were fed the standard (high-nutrient) diet (see Zera and Larsen, 2001). Crickets from generations 15–20 were used in biochemical characterizations and were raised under slightly lower density than those in the standard artificial selection experiment. This further reduced the frequency of SW females in the LW(f)-selected lines (to <10%), while retaining a high freq of SW females in SW-selected lines (>90%). Studies were conducted exclusively on females, both because of the labor intensive nature of the biochemical characterizations (performed on two-three days of adulthood, on nine lines, fed three diets), and because we were primarily interested in the biochemical basis of the trade-off between flight capability and early-age fecundity. The few (5–10%) SW females produced in the LW(f)-selected lines, or LW(f) females produced in the SW-selected lines were not characterized. In addition, by day 5 of adulthood, the last day on which crickets were compared for lipid characteristics, about 10% or less LW females had histolyzed their flight muscles (flight muscle histolysis is common in crickets and increases with age; see Zera *et al.*, 1997). These flightless females with long wings (designated LW[h]) are phenotypically very similar to SW females with respect to reproductive, physiological, and biochemical traits and are not considered here (*e.g.,* see Zera *et al.*, 1997; 1999; Zera and Larsen, 2001; Zera and Cisper, 2001; Zhao and Zera, 2001). Biochemical characterizations were performed on stocks fed a variety of diets (standard, low-nutrient, and high carbohydrate; see Zera and Larsen, 2001). However, data presented here were either pooled across the various diets or were obtained on the standard diet alone. Genetically-based differences in aspects of lipid metabolism were ascertained using paired *t*-tests, which compared the difference in the means of a trait between LW(f) and SW lines of a particular block, averaged across blocks (see Zera and Larsen, 2001; Zhao and Zera, 2002 and Table 1; also see Rose *et al.*, 1996). In essence, the paired *t*-test measures the consistency of differences between LW(f) and SW-selected lines across blocks. In some cases (*e.g.,* Zera and Zhao, 2003*b*), LW(f) and SW lines were crossed and backcrossed to document co-segregation between various traits. See Zera and Cisper (2001) and Zera and Larsen (2001) for additional details of the selection experiment and genetic analyses.

Differences in lipid levels between flight-capable (LW[f]) and flightless (SW) morphs

Triglyceride and phospholipids were measured in genetic stocks of *G. firmus* nearly pure-breeding for LW(f) (flight-capable) or SW (flightless) morphs (data for one block on the standard diet are presented in Fig. 3; see Zera and Larsen [2001] for data on all blocks and diets). The LW(f) morph exhibited a genetically-based, greater accumulation of triglycer-

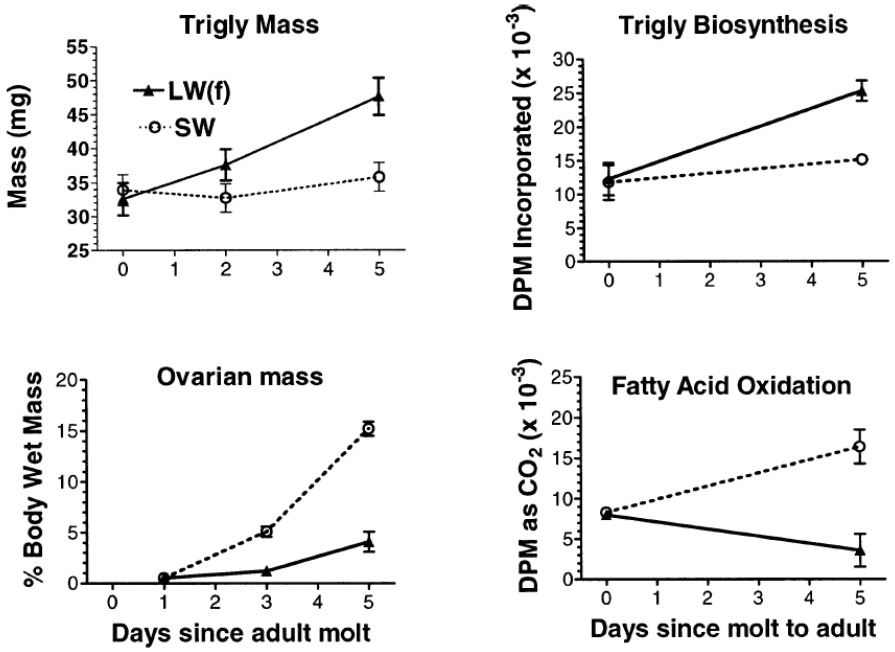

Figure 3. Relationships among whole-body triglyceride mass (top, left), ovarian mass (bottom, left), rate of triglyceride biosynthesis (top, right), and rate of fatty acid oxidation (bottom, right), during the first week of adulthood in LW(f) and SW adult *G. firmus*. Data are from LW(f) and SW lines of one representative block (Block 3) of the selection experiment (data from Zera and Larsen, 2001; Zhao and Zera, 2002; Zera and Zhao, 2003*a*). Differences of similar magnitude were observed between LW(f) and SW-selected lines of the other two blocks (see above references and text). Whole-body triglyceride masses were adjusted to whole-body, fat-free dry mass by ANCOVA. "Y" axes of triglyceride biosynthesis and fatty acid oxidation graphs represent amounts of radioactivity (DPM) incorporated into triglyceride or CO2 during a standard period of time (see text, Zhao and Zera [2002], and Zera and Zhao [2003*a*] for experimental details).

ide than the SW morph on each of several diets that differed in total calories, carbohydrate, or lipid content. Enhanced accumulation of triglyceride in the LW(f) morph occurred during the first week of adulthood, precisely when ovarian growth was substantially reduced relative to the SW morph (Fig. 3). Furthermore, enhanced accumulation of triglyceride was associated with decreased accumulation of phospholipid (Zera and Larsen, 2001). Thus, in *G. firmus*, there is a strong trade-off between triglyceride accumulation and ovarian growth, or phospholipid accumulation (Zera and Larsen, 2001). These morph-specific differences make functional sense because triglyceride is the main flight fuel in *Gryllus* (Zera *et al.*, 1999), while phospholipid is an important component of vitellogenin (yolk protein) (Beenakkers *et al.*, 1985). In addition to these whole-organism differences in triglyceride and phospholipid, the LW(f) morph allocates a disproportionately greater amount of whole-organism triglyceride and phospholipid to somatic (non-ovarian) tissues, while the SW morph allocates a disproportionately greater amount to the ovaries (A. J. Zera, unpublished data). As mentioned previously, lipid is important for reproduction as well as for flight, and eggs have high triglyceride and phospholipid contents (Beenakkers *et al.*, 1985). The substantially greater amount of total lipid and triglyceride found in the dispersing morph, of *G. firmus* and other dispersal-polymorphic species (Nwanze *et al.*, 1976; Gunn and Gatehouse, 1993), indicates that lipid requirements are greater for dispersal than for reproduction during early adulthood.

Lipid acquisition from the diet does not differ between LW(f) and SW morphs

The substantial differences in lipid levels between LW(f) and SW-selected lines of *G. firmus* (Zera and Larsen, 2001) could result from morph-specific differences in any of several aspects of lipid metabolism (*e.g.,* biosynthesis or oxidation). Alternatively, increased accumulation of lipid in the LW(f) morph during the first week of adulthood could simply result from greater nutrient intake (*i.e.,* increased consumption and/or assimilation), rather than morph-specific differences in internal metabolism. Surprisingly, most recent physiological-energetic studies life-history trade-offs have not quantified or controlled for potential differences in nutrient input between life history phenotypes (*e.g.,* nearly all studies of *Drosophila*; Zera and Harshman, 2001).

We conducted extensive feeding studies in three *Gryllus* species and documented that, in each case, flight-capable and flightless females consume the same, or nearly the same (within 10%), amount of food and do not differ in assimilation of total nutrients, lipid, carbohydrate, or protein when fed the standard diet (Mole and Zera, 1993; Zera *et al.*, 1998; Zera and Brink, 2000; A. J. Zera, unpublished data). (Note: Significantly-reduced consumption by flightless *vs.* flight-capable females

of *G. firmus* was originally reported by Mole and Zera [1994]. However, that result was due to abnormal retention of eggs by the more fecund SW females, which compressed their gut and inhibited feeding during the second week of the two-week feeding trial. Subsequent studies, restricted to the first week of adulthood, which is the same period of time during which lipid studies were conducted, demonstrated that mean consumption was only 10% lower in LW(f) *vs.* SW females [A. J. Zera, unpublished data].) Thus, elevated lipid levels in LW(f) *vs.* SW females must result exclusively from differential metabolism of internal nutrients rather than from differential acquisition of nutrients from the diet. *G. firmus* is one of the only insects for which differential acquisition of nutrients can be eliminated as a potential cause of phenotypic differences in energy reserves.

LW(f) and SW morphs differ in rates of lipid biosynthesis and oxidation

In vivo rates of total lipid, triglyceride and phospholipid biosynthesis were compared between LW(f) and SW morphs by quantifying the amount of radiolabelled precursor (^{14}C-acetate or ^{14}C-palmitic acid) incorporated into these lipids (see Zhao and Zera [2002] and the legend of Figure 4 for experimental details). Figure 4 (upper panel) illustrates a schematic diagram of the *de novo* pathways of triglyceride, and phospholipid biosynthesis. Briefly, fatty acids, most commonly 16–18 carbons in length (Beenakkers *et al.*, 1985; Grapes *et al.*, 1989), are synthesized from acetate (acetyl-CoA). These fatty acids are then combined with glycerol phosphate, and converted into either triglycerides or phospholipid, which comprise greater than 90% of total lipid in *Gryllus* and most insects (Beenakkers *et al.*, 1985; Grapes *et al.*, 1989; Zera and Larsen, 2001). Thus, biosynthesis of triglycerides and phospholipids consists of two parts: (1) the production of fatty acids that comprise the bulk (> 90%) of these molecules, and (2) the partitioning of fatty acids into these two lipid classes.

On the first day of adulthood, rates of triglyceride or phospholipid biosynthesis were low and equivalent in LW(f) and SW females (Zhao and Zera, 2002), consistent with the low and equivalent levels of these compounds in LW(f) and SW morphs found on day 0 (Fig. 3; Zhao and Zera, 2002). By contrast, on day 5 of adulthood, we observed a substantially higher rate of triglyceride biosynthesis and lower rate of phospholipid biosynthesis in LW(f) *vs.* SW selected lines (Figs. 3 and 5). No interactions were observed between diet or line type (LW[f] *vs.* SW) and data in Figure 5 are biosynthetic rates pooled across the three diets. These results are consistent with an increased rate of triglyceride biosynthesis, and a decreased rate of phospholipid biosynthesis being important factors that cause the greater accumulation of triglyceride and lesser accumulation of phospholipid in the LW(f) *vs.* SW morphs (Fig. 3; Zhao and Zera, 2002). Finally, the elevation in triglyceride biosynthesis was much greater than the decrease in phospholipid biosynthesis in the LW(f) morph. Thus, biosynthesis of total lipid (= total fatty acid) was higher in LW(f) *vs.* SW females (data not shown; see Table 1 of Zhao and Zera, 2002).

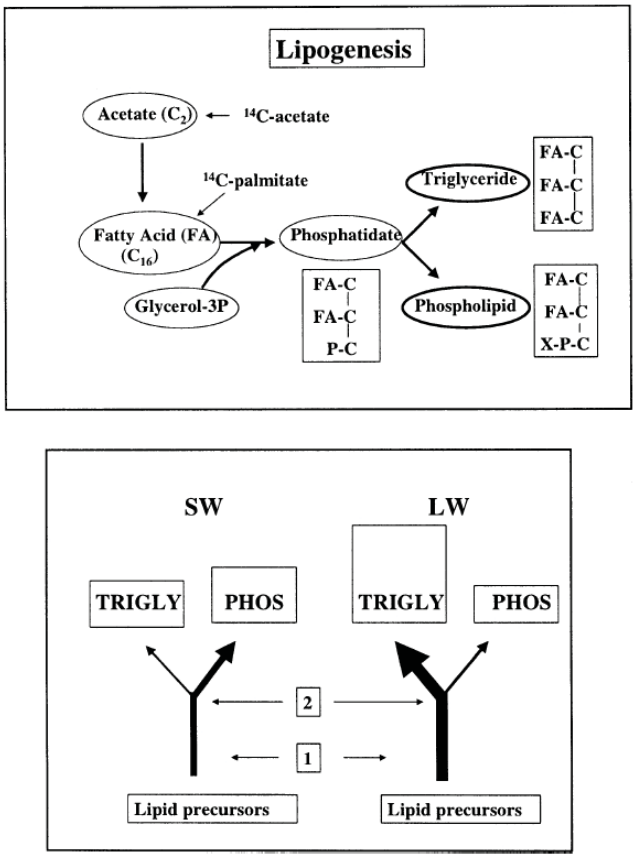

Figure 4. *Top panel.* Simplified pathway of *de novo* biosynthesis of triglyceride and phospholipid from acetate (see Downer, 1985). Glycerol-3P 5 glycerol-3-phosphate. Box under phosphatidate indicates that this compound is composed of two fatty acids (FA) linked to glycerol-3-phosphate. Boxes next to triglyceride and phospholipid indicate that these compounds are produced from phosphatidate by removal of the phosphate group (to produce diglyceride) and subsequent addition of (1) a third fatty acid (triglyceride), or (2) a phosphorylated compound such as phosphocholine (phospholipid). ^{14}C-acetate, next to acetate, and ^{14}C-palmitate, next to fatty acid, indicate that radiotracer studies of lipid biosynthesis were conducted by injection of either radio-labelled acetate or palmitate into whole crickets. Amount of radiolabel incorporated into triglyceride and phospholipid during a given period of time was subsequently quantified. See Zhao and Zera (2001, 2002) for experimental details.

Bottom panel. Morph-specific trade-offs in glyceride (triglyceride *vs.* phospholipid) biosynthesis in *G. firmus* identified in radiotracer studies of Zhao and Zera (2002). "Y" diagrams illustrate relative flow of ^{14}C through *de novo* pathway of fatty acid biosynthesis in LW(f) (denoted as "LW" in this figure) and SW females. Total glyceride biosynthesis (=total fatty acid biosynthesis; denoted by width of base of "Y") is greater in LW(f) than in SW females. Total and relative biosynthesis of triglyceride is greater in LW(f) than in SW females, while total and relative biosynthesis of phospholipid is greater in SW than in LW(f) females. Widths of lines, which are only meant to illustrate rank-order and not quantitative differences between morphs, are based on data from Table 1, Figure 5, and additional data from Zhao and Zera (2002).

Because the biosynthesis of triglyceride and phospholipid was measured in each individual, we also could estimate

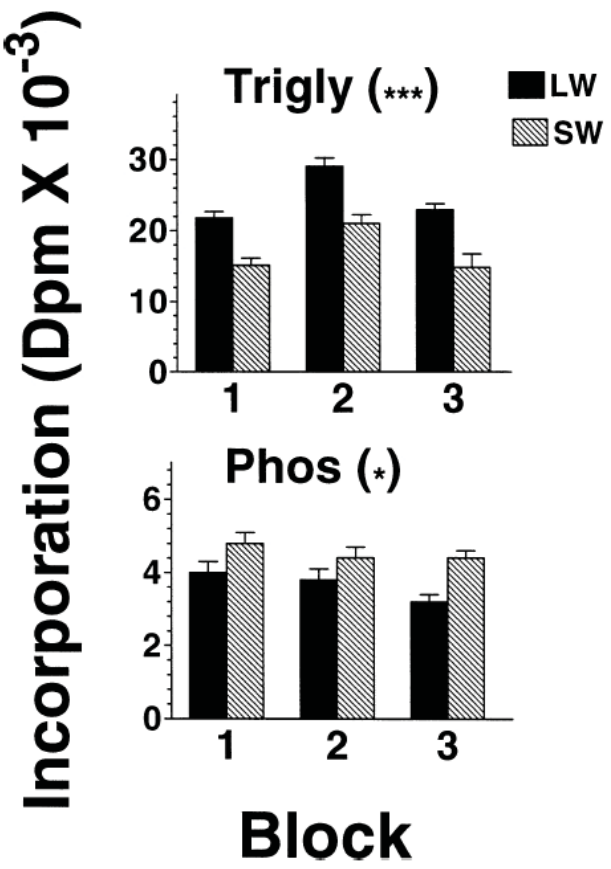

Figure 5. Rate of biosynthesis of triglyceride and phospholipid from radiolabelled acetate by LW(f) and SW-selected lines of *G. firmus* on day 5 of adulthood (DPM [radioactivity] incorporated during a seven-hour incubation period). Histograms refer to means (±SEM) of LW(f) or SW genetic stocks from the 3 blocks (data from Zhao and Zera, 2002). Asterisks refer to results of paired *t*-tests of LW and SW line means (*** = $P < 0.005$, * = $P < 0.05$). Note the higher rate of biosynthesis of triglyceride but lower rate of biosynthesis of phospholipid in LW(f) *vs.* SW selected lines of *G. firmus*. Similar results were obtained when ^{14}C-palmitate was the radiolabel (see Zhao and Zera, 2002).

the proportional biosynthesis of these two key lipid classes via ANCOVA. Results presented in Table 1 indicate a genetically based trade-off in the biosynthesis of these two lipid classes. That is, relative to SW-selected lines, LW(f) selected lines consistently converted a *proportionately* greater amount of acetate or palmitate into triglyceride. Thus, two important, genetically-based alterations in lipid biosynthesis were observed in selected lines of *G. firmus* (Fig. 4, lower panel). First, LW(f) lines exhibited increased flux through the *de novo* pathway of fatty acid biosynthesis (resulting in the biosynthesis of a greater amount of fatty acid and hence total lipid). Second, there was a more downstream trade-off involving the greater diversion of fatty acids into triglyceride *vs.* phospholipid in the LW(f) morph. Even though the rate of total lipid biosynthesis was lower in the SW morph, the greater diversion of fatty acid into phospholipid resulted in a higher rate of phospholipid biosynthesis in this morph, compared with the LW(f) morph. As mentioned above, phospholipid is an important component of vitellogenin, and plays an important role in embryo development (Beenakkers *et al.*, 1985). To our knowledge, this represents the first direct documentation of genetically-based alterations in the *in vivo* flux through pathways of intermediary metabolism leading to the differential production of end products central to the specialization of phenotypes for alternate life histories (Zhao and Zera, 2002).

In addition to its genetically-based elevation in total lipid and triglyceride biosynthesis, the LW(f) morph also exhibited a genetically-based reduction in the rate of fatty acid oxidation relative to its SW counterpart (Figs. 3 and 6, upper panel). Rate of fatty acid oxidation was measured as the amount of radiolabelled, injected fatty acid (palmitate) or acetate that was converted into CO_2 during a standard incubation period (see Zera and Zhao, 2003*a* for experimental details). On the day of emergence, the rate of fatty-acid oxidation was equivalent in the LW(f) and SW morphs, similar to the situation for lipid biosynthetic rate. However on day 5, the rate of fatty acid oxidation was significantly higher in the SW-selected line relative to the LW(f) line of the same block for each of the three blocks. This was the case for lines fed each of the three diets (see Zera and Zhao, 2003*a*). As was the case for lipid biosynthesis, no interaction between line type and diet was observed, indicating that differences between the morphs in fatty acid oxidation were roughly equivalent on each of the three diets.

A genetically-based trade-off was observed in the proportion of fatty acid (or acetate) that was oxidized *vs.* converted into glycerides (*i.e.,* triglyceride and phospholipid) (Fig. 6, lower panel; Zera and Zhao, 2003*a*). Thus, the elevated triglyceride reserves in the LW(f) morph resulted from a decreased rate of fatty acid oxidation, in addition to an elevated rate of glyceride biosynthesis. By contrast, the elevated rate of fatty-acid oxidation in the SW morph reduces the availability of precursors for glyceride biosynthesis, but probably contributes a significant amount of energy necessary for the enhanced biosynthesis of vitellogenin (yolk protein) in SW females. Recent studies indicate a complimentary situation for amino acid metabolism in *G. firmus*: LW(f) females oxidize a greater amount of amino acids than do SW females, which likely reduces yolk protein production in LW(f) females, but which contributes energy to drive the enhanced biosynthesis of fatty acid for subsequent conversion to triglyceride flight fuel (A. Zera and Z. Zhao, unpublished data).

Morphs differ in the allocation of biosynthesized triglyceride and phospholipid to somatic vs. reproductive organs

In addition to measuring whole-organism biosynthesis and oxidation of triglyceride and phospholipid, we also measured, in a separate experiment, the amounts of newly biosynthesized triglyceride and phospholipid that were allocated to somatic *vs.* reproductive (ovarian) tissue in selected lines of one block (Fig. 7; Zhao and Zera, 2002). Not only did the LW(f) morph biosynthesize a significantly greater amount of triglyceride than did the SW morph (as in the previous experiment discussed

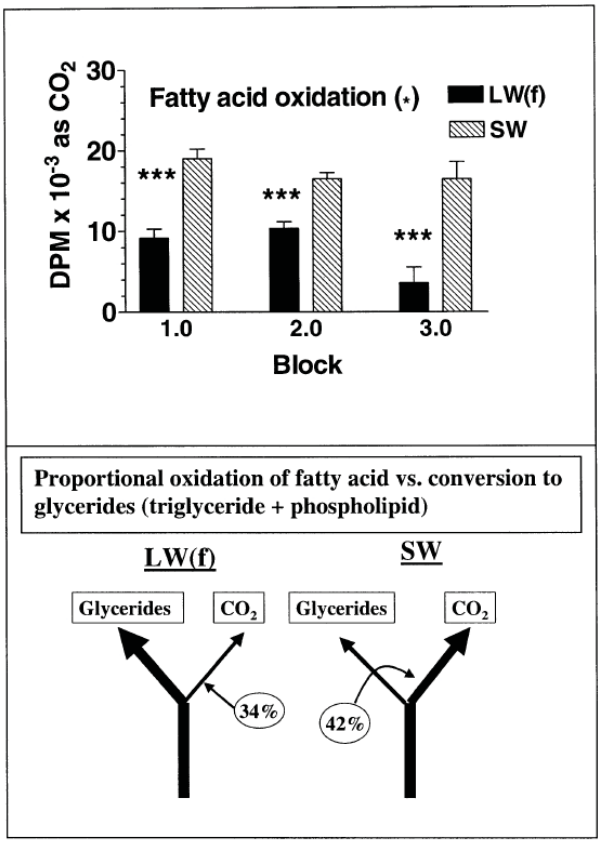

Figure 6. *Top panel.* Rate of fatty acid oxidation in LW(f) and SW-selected lines of *G. firmus* on day 5 of adulthood raised on the standard (high) diet. Histograms refer to the mean (±SEM) amount of injected ^{14}C-palmitic acid converted into ^{14}C-CO_2 during the standard four-hour incubation period. Asterisks above the histograms refer to results of *t*-tests of LW(f) and SW individuals within a block, while asterisk within the parenthesis refer to results of paired *t*-test comparing line means across blocks (*** = $P < 0.005$; * = $P < 0.05$). Note the consistently greater rate of fatty acid oxidation in SW *vs.* LW(f) lines. *Bottom panel.* Genetically-based trade-off between LW(f) and SW lines with respect to the proportional oxidation of fatty acid *vs.* utilization for glyceride (triglyceride and phospholipid) biosynthesis. Values represent mean percentage of radiolabelled ^{14}C palmitic acid that was oxidized to CO_2 *vs.* oxidized into CO_2 plus incorporated into glycerides for a representative pair of LW(f) and SW lines. These mean percentages differ significantly as do values for LW(f) and SW lines from the other two blocks (Results of ANCOVAs: $P < 0.005$ in each case; see Zera and Zhao, 2003*a* for statistical analyses).

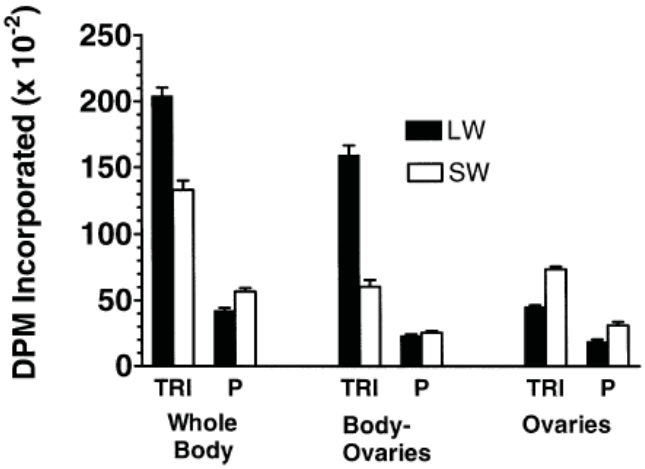

Figure 7. Allocation of biosynthesized triglyceride and phospholipid into reproductive (ovaries) *vs.* somatic (whole-body minus ovaries) tissues in *G. firmus*. Data are means ± SEMs for lines of Block 2 (Zhao and Zera, 2002). TRI = triglyceride, P = phospholipid. The LW(f) morph both biosynthesizes significantly more triglyceride than the SW morph and allocates significantly more to somatic tissue. The SW morph biosynthesizes significantly more phospholipid and allocates significantly more to ovarian tissue. See text and Zhao and Zera (2002) for results of statistical analyses.

above), it also allocated a disproportionately greater amount of triglyceride to somatic *vs.* ovarian tissue (Fig. 7). Conversely, the SW morph both biosynthesized more phospholipid and allocated more of this lipid class to ovarian tissue. These results have important implications for energetic studies of life history trade-offs. For example, they illustrate the degree to which precision in the estimate of energy devoted to the soma *vs.* reproduction can be increased when individual lipid components are measured in individual body compartments. Just to give one example of this point, LW(f) females biosynthesized 60% more *whole-body* triglyceride compared with SW females (Zhao and Zera, 2002). Assuming that whole-body triglyceride has an exclusively somatic function, a common assumption in insect life history studies (Zera and Harshman, 2001), one would conclude from these data that there is a 60% greater lipid allocation to somatic function in LW(f) *vs.* SW females. However, this turns out to be a substantial underestimate. Using biosynthesized triglyceride that is actually found in the somatic body compartment (*i.e.,* non-ovarian tissues), a 200% increased allocation to somatic lipid is observed in LW(f) females. The difference between these two estimates is due to the former estimate not taking into account the disproportionately reduced allocation of whole-body triglyceride to the soma in the SW morph. Indeed, the majority of whole-body triglyceride in the SW morph is found in the ovaries (Zhao and Zera, 2002). Estimating allocation of triglyceride to reproductive *vs.* somatic functions is likely to be even more complex, because the ovarian and fat-body triglyceride pools are probably dynamic and interconvertible to some degree.

LW(f) and SW morphs differ in specific activities of lipogenic enzymes

In vitro specific activities of a representative group of enzymes involved in lipid biosynthesis were compared between LW(f) and SW-selected lines. Activities were measured in fat body, the most important lipogenic organ in insects (Downer, 1985). Names and metabolic roles of enzymes are given in Figure 8, while specific activities measured on crickets fed

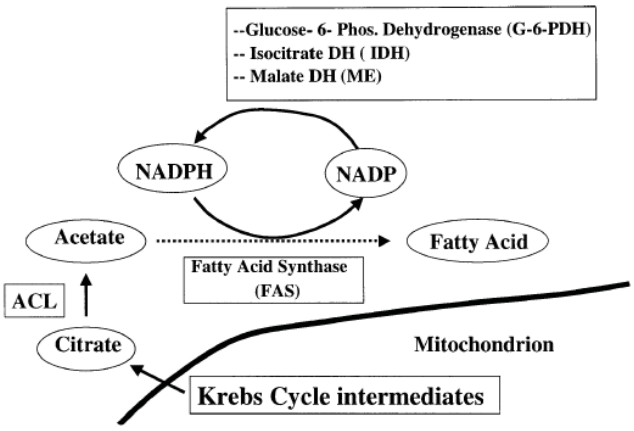

Figure 8. Enzymes of the *de novo* pathway of fatty-acid biosynthesis (Downer, 1985) whose activities were compared between LW(f) and SW morphs. ACL (ATP-citrate lyase) converts citrate, transported outside the mitochondrion, into acetate (=acetyl CoA). Fatty acid synthase (FAS) is a complex enzyme that converts malonyl CoA (produced from acetyl CoA in the preceding step in the pathway) into a 16 carbon fatty acid through a series of chemical reactions denoted by the dotted line. Reducing equivalents (NADPH) required for fatty acid biosynthesis are produced from NADP by the enzymes glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, and malate dehydrogenase.

the standard diet are given in Figure 9 (see Zera and Zhao, 2003*b* for additional enzymes, assays performed on crickets fed different diets, and assay conditions). Lipogenic specific activities were measured to independently assess whether lipid biosynthesis is elevated in the LW(f) morph, and to identify specific enzymes involved (Zera and Zhao, 2003*b*). Specific activity of *each* lipogenic enzyme studied was substantially elevated genetically in LW(f) *vs.* SW lines (Fig. 8; Zera and Zhao, 2003*b*). Thus, the genetic differences in fatty acid biosynthesis between LW(f) and SW morphs appear to result from a global alteration of the activities of enzymes involved in lipid biosynthesis rather than from modifications of a few "key" enzymes. *In vivo* differences in activities of these enzymes between LW(f) and SW morphs, are even greater than indicated in Figure 9. This is because there is about a 40% greater amount of fat body in the LW(f) compared with the SW morph (on the standard diet), and activities in Figure 9 were standardized to the same amount of fat body protein (*i.e.,* they are specific activities). Line-crosses and backcrosses documented strong co-segregation among activities of each of these enzymes, wing length, flight muscle mass, and ovarian mass (Zera and Zhao, 2003*b*).

Metabolic differences between LW(f) and SW lines result from differences in endocrine regulation

LW(f) and SW-selected lines differ with respect to a wide variety of biochemical traits (summarized in Table 2), as well as various morphological and reproductive traits (*e.g.,* size of wings, flight muscles, ovaries, and fat body; Zera *et al.*, 1997; Zera and Zhao, 2003*b*, 2004). The co-ordinate expression of these alternate sets of diverse traits in LW(f) and SW-selected lines likely results from genetic differences in endocrine

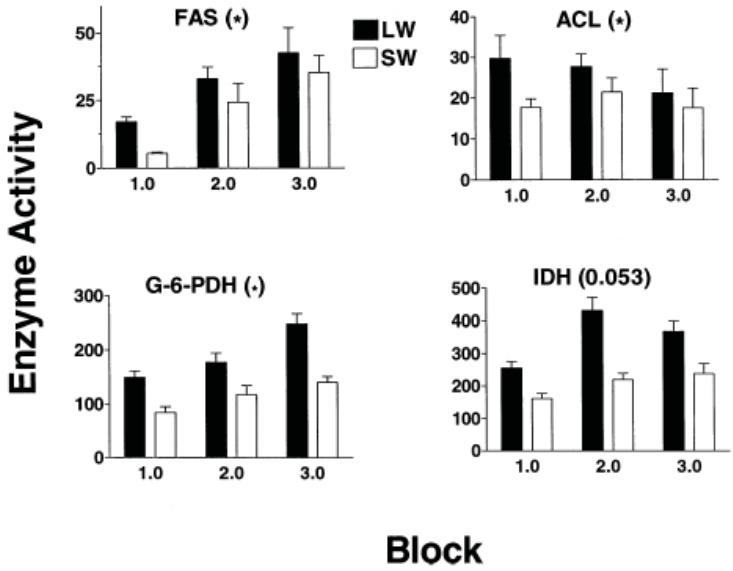

Figure 9. Specific activities (nmol min^{-1} mg $protein^{-1}$) of representative lipid-biosynthesizing enzymes from fat body of LW(f) and SW-selected lines of *G. firmus* that were fed the standard diet. Activities (means ± SEM) were measured on day 5 of adulthood (data from Zera and Zhao, 2003*b*). See legend of Figure 8 for names of enzymes. Asterisks in parentheses denote results of paired *t*-tests. See Zera and Zhao (2003*b*) for activities of other lipogenic enzymes and activities measured on other diets. Note the higher activity of each enzyme in LW(f) compared with SW-selected lines in each block.

TABLE 2. *Genetic differences in lipid metabolism between flight-capable and flightless morphs of* G. firmus.

Aspect of metabolism	Flight capable (LW(f)) morph relative to flightless (SW) morph	References
Biosynthesis of total lipid	Higher	A, B
Absolute biosynthesis of triglyceride	Higher	A
Absolute biosynthesis of phospholipid	Lower	A
Relative biosynthesis of triglyceride/phospholipid	Higher	A
Oxidation of fatty acid	Lower	C
Relative utilization of fatty acid for biosynthesis vs. oxidation	Higher	C
Specific activities of lipogenic enzymes	Higher	B, D
Conversion of amino acids into lipid	Higher	E
Oxidation of amino acid	Higher	E
Activity of transminases	Higher	D, B
Biosynthesis of ovarian protein from amino acids	Lower	E
Allocation of biosynthesized lipid to soma vs. ovaries		
Triglyceride	Higher	A
Phospholipid	Lower	A
Assimilation of lipid from diet	Equivalent	F

Characteristics of individuals reared on the "standard" diet (See Zera and Larsen [2001] for diet composition). Flight capable = long-winged individuals with large pink (functional) flight muscles; flightless = short-winged individuals with underdeveloped flight muscles. References: A = Zhao and Zera, 2002; B = Zhao and Zera, 2001; C = Zera and Zhao, 2003*a*; D = Zera and Zhao, 2003*b*; E = A. J. Zera and Z. Zhao unpublished data; F = Zera and Brink, 2000.

regulation. One potential candidate regulator is juvenile hormone (JH), a hormone of central importance in insects (Nijhout, 1994; Wyatt and Davey, 1996). JH positively affects ovarian growth, and negatively affects many aspects of flight-capability such as the size of wings, flight muscle mass, fat body mass, and lipid accumulation in many insects including *Gryllus* (Nijhout, 1994; Zera *et al.*, 1998; Zera, 2004). Thus, a genetically-specified difference in the titer of, or tissue sensitivity to, JH could account for the differential co-expression of the various biochemical, morphological, and reproductive traits observed in SW and (LW[f])-selected lines.

Although the hormonal control of intermediary metabolism has been extensively studied in vertebrates (*e.g.,* Granner and Pilkis, 1990; Sul and Wang, 1998), the endocrine regulation of intermediary metabolism is much less understood in insects. As a first step in determining whether differences in the expression of morph-specific traits between selected lines result from differences in endocrine regulation, we applied methoprene, a juvenile hormone analogue, to adult females of one of the LW(f) selected lines (Zera and Zhao, 2004). This manipulation produced a remarkable SW phenocopy with respect to numerous reproductive, anatomical, and biochemical traits (Fig. 10). For example, relative to LW(f) controls, LW(f) females treated with methoprene had larger ovaries, decreased flight-muscle mass, decreased rate of triglyceride biosynthesis, decreased specific activities of lipogenic enzymes, increased rate of fatty acid oxidiation, and increased rate of phospholipid biosynthesis, all characteristics of the SW morph (Fig. 10; Zera and Zhao, 2004). Remarkably, hormonally-treated LW(f) and control LW(f) females differed in these traits to the same degree as did untreated SW and LW(f) females (Fig. 10; Zera and Zhao, 2004). These results provide strong evidence that morph-specific differences in lipid metabolism are caused by variation in endocrine regulation. However, differences in the *in vivo* JH titer between LW(f) and SW morphs are complex (Zera and Cisper, 2001; Zhao and Zera, 2004), and it is presently unclear whether JH itself or other hormones regulate differences in lipid metabolism between the morphs. For example, the blood titer of ecdysteroids, a group of hormones that regulates numerous traits in insects (Nijhout, 1994), also differs substantially between LW(f) and SW adult *G. firmus* (Zera and Bottsford, 2004; Zhao and Zera, 2004), and the titer of this hormone may be strongly altered by topical application of methoprene (Zera, 2004). Thus differences in the titer of ecdysteroids (or other as yet unidentified hormones) may regulate the morph-specific differences in lipid metabolism.

SUMMARY AND SYNTHESIS

Modifications of intermediary metabolism, that lead to changes in the production of key life-history components, such as yolk protein for eggs, triglyceride for somatic maintenance and dispersal, and energy for growth and metabolism, almost certainly are key components of life history evolution. A deep understanding of the mechanisms underlying life-history evolution thus requires detailed knowledge of these modifications. For example, only by identifying these alterations in intermediary metabolism can the chain of causality be traced from variation in DNA sequence to variation in the phenotypic expression of whole-organism life histories. Detailed information on variation within and interactions among metabolic pathways will be required to understand many central issues in life-history evolution such as the nature and stability of genetic correlations between life-history traits and the nature of constraints in life-history evolution (see O'Brien *et al.* [2002] for a recent excellent example of this latter point).

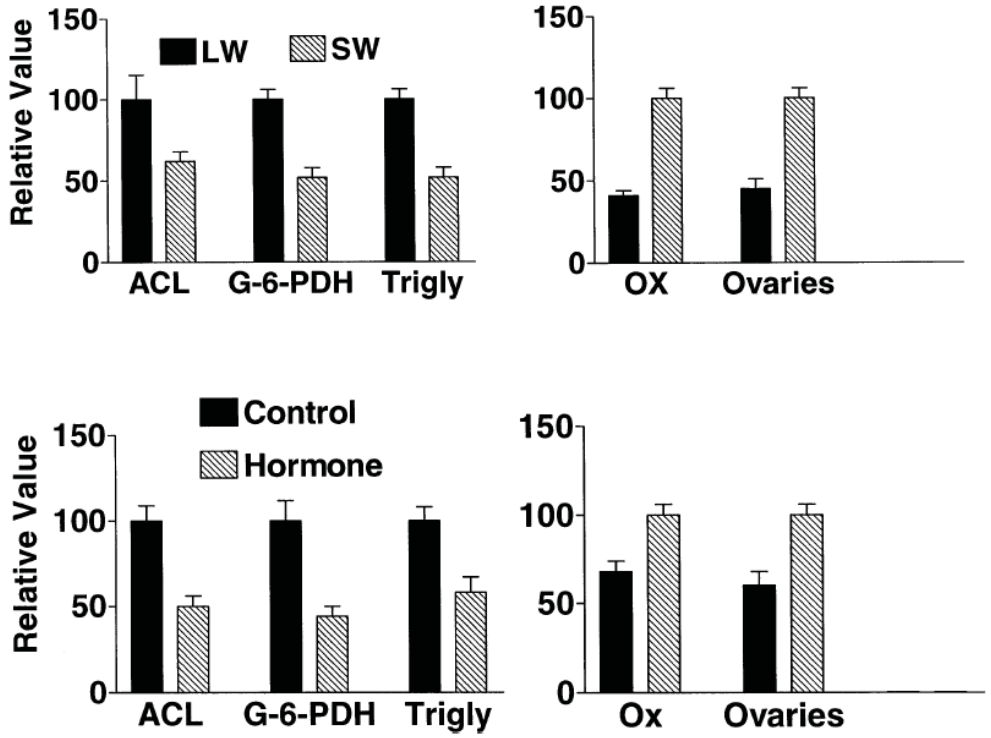

Figure 10. Production of a SW biochemical and reproductive phenocopy by topical application of a juvenile hormone analogue (methoprene) to LW(f) *G. firmus*. *Bottom panels:* Relative decrease in specific activities of lipid biosynthetic enzymes (ACL, and G-6-PDH) and rate of triglyceride biosynthesis (Trigly) (left), and relative increase in rate of fatty acid oxidation (OX) and ovarian mass (Ovaries) (right) in hormone-treated LW(f) (Hormone) *vs.* untreated (Control) females on day 5 of adulthood. *Top panels:* Relative decrease or increase in the same traits in unmanipulated SW *vs.* unmanipulated LW(f) (denoted as "LW" in this figure) females on day 5 of adulthood. Note the similar magnitude of increase or decrease in aspects of lipid metabolism and ovarian mass in hormone-treated (SW phenocopy) *vs.* untreated LW(f) crickets (bottom panels) as are seen in untreated SW *vs.* untreated LW(f) crickets (top panels). All comparisons between untreated LW(f) and untreated SW morphs or between treated or control LW(f) individuals were statistically significant ($P < 0.01$). See legend of Figure 8 for full names of enzymes. Data are for crickets from LW(f) and SW selected lines of block 2, raised on the standard diet (Zera and Zhao, 2004). See Zera and Zhao (2004) for units of the various assays and additional data measured on other diets. In this figure, data were scaled such that "100" for the control refers to the actual value reported for the LW(f) control in Zera and Zhao (2004) with the value for the hormone-treated LW(f) morph expressed relative to that value.

Recent studies of lipid metabolism in selected lines of *G. firmus*, described above, currently represent the most detailed investigations of alterations in intermediary metabolism underlying a naturally-occurring, genetically-based life history trade-off (Table 3; Zhao and Zera, 2002; Zera and Zhao, 2003*a*, *b,* 2004). Most notably, recent studies in *G. firmus* have directly documented that the differential flow of metabolites through specific pathways of intermediary metabolism underlie an internal, resource-based trade-off important for morph specialization. That is, the SW and LW morphs differ genetically in the degree to which fatty acids are (1) oxidized for energy *vs.* used for glyceride biosynthesis, and (2) partitioned between triglyceride and phospholipid biosynthesis (Zhao and Zera, 2002; Zera and Zhao, 2003*a*). These trade-offs result in enhanced production of triglyceride flight fuel in the LW(f) morph, and enhanced production of phospholipid and energy in the SW morph. These two functionally-important, biochemical trade-offs are now useful models to investigate the molecular and hormonal regulation of resource-allocation trade-offs. More recent studies have identified additional, functionally-important interactions and trade-offs between amino-acid and lipid metabolism in LW(f) and SW females (A. Zera and Z. Zhao, unpublished data). These studies collectively illustrate the remarkable alterations in intermediary metabolism that underlie the evolution of alternate life histories in dispersing *vs.* reproductive morphs of *G. firmus*.

Another important finding of the *Gryllus* studies is that differential flux through the pathway of lipid biosynthesis in LW(f) and SW morphs appears to result from substantial alterations in the activities of many enzymes of lipogenesis (Figs. 8–9; see Zera and Zhao [2003*b*] for activities of other enzymes). This finding is similar to results of other recent artificial or laboratory selection studies, in which changes in carbohydrate or lipid metabolism are associated with alteration of activities or mRNA abundance of many pathway enzymes (*e.g.,* Clark *et al.*, 1990; Asante *et al.*, 1991; Ferea *et al.*, 1999). Similarly, changes in pathway flux due to hormonal regulation (*e.g.,* changes in glycolysis and glucoeneo-

genesis modulated by insulin or glucagon) also appear to result from co-ordinate modulation of many pathway enzymes (*e.g.,* Granner and Pilkis, 1990), termed "multisite modulation" (Fell, 1997). Thus, short-term or evolutionary changes in flux through metabolic pathways, appear to require co-ordinate alteration of many enzymes, a finding that is consistent with theoretical expectations of Metabolic Control Theory (Kascer and Burnes, 1979; Fell, 1997). These observations further suggest that hormones (or other regulators) that co-ordinate the expression of multiple enzymes of a pathway are likely to be particularly important targets of selection on pathway flux (Ferea *et al.*, 1999; Zera and Zhao, 2004).

Thus, another important finding of the *Gryllus* studies is that alteration of hormonal regulation appears to be an important cause of morph-specific differences in lipid metabolism (Fig. 10; Zera and Zhao, 2004). Although hormones have been implicated as causal factors in life history trade-offs (Ketterson and Nolan, 1999; Sinervo, 2000; Zera and Cisper, 2001; Zera and Harshman, 2001), they have typically not been invoked to explain differential allocation of resources (Zera and Zhao, 2003*b*). Indeed, some workers have viewed differential resource allocation *vs.* differential regulation as alternate explanations for life history variation and trade-offs (Rose and Bradley, 1998; Leroi, 2001). This is reminiscent of the previous dichotomization of "structural" *vs.* "regulatory" changes as being the most important causes of molecular evolution (*e.g.,* Wilson, 1976). An important message of the *Gryllus* studies is that modification of endocrine regulation may be the primary cause of differential resource allocation, which, in turn, is a key component of the trade-off between early-age reproduction and flight-capability in *G. firmus*.

Finally, these studies illustrate the power of wing polymorphism in *Gryllus* as an experimental model to investigate the functional causes of life history variation and trade-offs (Zera and Harshman, 2001; Zera and Zhao, 2003*b*, 2004). Morph-specific differences in life histories are of sufficient magnitude, and crickets are large enough, to allow analyses of the physiological, biochemical, and endocrine causes of life history variation to be studied in individual organs (*e.g.,* organ-specific enzyme activities). Highly significant genetic differences were documented between morphs of *G. firmus* in numerous biochemical traits, even though statistical power was low (2 df in paired *t*-tests; = [number of blocks] − 1). Because of the extensive data on variation in lipid metabolism and covariation with life history traits, wing polymorphism is now an especially useful experimental model to investigate the detailed molecular and endocrine mechanisms underlying the microevolution of metabolism and life histories.

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