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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

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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 Cisneros, 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).

*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).

Artificial selection is a powerful tool to investigate the mechanisms of evolution (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Gibbons, 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;...
and reproduction. To put these studies in context I id metabolism that contribute to the trade-off between
ies has been to identify genetically-based alterations in lip-
ship between lipid physiology and life history trade-offs.
background information on wing polymorphism, and the rela-
lected to produce morphs adapted for the
of the wing-polymorphic cricket, Gryllus firmus, artificially
polymorphism is common in many insect groups, most notably the He-
phism is the most dramatic example of the trade-off between
flight at the expense of reproduction, and vice versa. The ultimate goal of these stud-
has been to identify genetically-based alterations in lip-
product of this paper. The middle and right panels illustrate dissections of morphs showing much larger, func-
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.

Zera and Zhao, 2003a, b). This review primarily focuses on re-
sults 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 stud-
ies has been to identify genetically-based alterations in lip-
id 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 rela-
tionship 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 polymor-
phism is common in many insect groups, most notably the He-
miptera/Homoptera (waterstriders, planthoppers and aphids),
Coleoptera (beetles) and, Orthoptera (crickets and grasshop-
pers) (Johnson, 1969; Harrison, 1980; Roff, 1986; Dingle,
1996; Zera and Denno, 1997). Natural populations of the crick-
et, 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-de-
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 el-
evated fecundity (100–400%) relative to the LW(f) morph (Fig.
1; Zera and Denno, 1997; Zera and Harshman, 2001; see be-
low). 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 appar-
atus), rather than flight itself, on egg production. Wing polymor-
phism 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; Harri-
son, 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 arti-
ficial selection (Roff, 1990; Zera and Cisper, 2001; Fig. 2).
Environmental factors such as density, photoperiod, temper-
ature, and food quality, also strongly affect morph expres-
sion 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, indi-
cate that wing polymorphism is maintained by environmental
heterogeneity (Vepsalainen, 1978; Denno et al., 1980, 1991;
Roff, 1994; Dingle, 1996; Zera and Denno, 1997). With-
in a patch, the more-fecund flightless morph has a substan-

**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.
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 McFarlane, 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 (Granmer and Pilkis, 1996; Sul and Wang, 1998). Yet we are almost completely ignorant of alterations in the endocrine control of
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-selected lines across blocks. In some cases (e.g., Zera and Zhao, 2003b), 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.

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

<table>
<thead>
<tr>
<th>Line type</th>
<th>Block</th>
<th>Result of paired t-test of line means</th>
</tr>
</thead>
<tbody>
<tr>
<td>LW(f)</td>
<td>1</td>
<td>84.2 ± 0.8 (26)**b</td>
</tr>
<tr>
<td>SW</td>
<td>2</td>
<td>83.6 ± 0.5 (22)**</td>
</tr>
<tr>
<td>RW(f)</td>
<td>3</td>
<td>86.3 ± 0.8 (24)**</td>
</tr>
<tr>
<td>SW</td>
<td>1</td>
<td>82.4 ± 1.9 (24)</td>
</tr>
<tr>
<td>RW(f)</td>
<td>2</td>
<td>79.1 ± 1.1 (24)</td>
</tr>
<tr>
<td>RW(f)</td>
<td>3</td>
<td>t&lt;sub&gt;D&lt;/sub&gt; = 7.38**</td>
</tr>
<tr>
<td>SW</td>
<td>1</td>
<td>69.7 ± 1.7 (20)**</td>
</tr>
<tr>
<td>RW(f)</td>
<td>2</td>
<td>64.5 ± 2.4 (21)**</td>
</tr>
<tr>
<td>RW(f)</td>
<td>3</td>
<td>67.7 ± 3.3 (22)**</td>
</tr>
<tr>
<td>SW</td>
<td>1</td>
<td>48.2 ± 2.6 (20)</td>
</tr>
<tr>
<td>RW(f)</td>
<td>2</td>
<td>46.9 ± 2.2 (23)</td>
</tr>
<tr>
<td>RW(f)</td>
<td>3</td>
<td>51.6 ± 2.4 (25)</td>
</tr>
<tr>
<td>SW</td>
<td>1</td>
<td>t&lt;sub&gt;D&lt;/sub&gt; = 4.90</td>
</tr>
<tr>
<td>RW(f)</td>
<td>2</td>
<td>P &lt; 0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are mean percentage (±SEM) of radioactivity of palmitate converted into (triglyceride + phospholipid + triglyceride) for the 3 pairs of LW(f) and SW-selected lines. Note the consistently greater biosynthesis of triglyceride vs. phospholipid in LW(f) vs. SW lines (Data from Zhao and Zera, 2002).

<sup>b</sup> Asterisks represent results of paired t-tests comparing LW(f) and SW morphs within a block.

<sup>c</sup> 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).

**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 triglyceri-
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 (14C-acetate or 14C-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).

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

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**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 phospholipid 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). 14C-acetate, next to acetate, and 14C-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 14C 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).
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 CO2 during a standard incubation period (see Zera and Zhao, 2003a 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, 2003a). 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, 2003a). 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
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
the standard diet are given in Figure 9 (see Zera and Zhao, 2003b 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, 2003b). Specific activity of each lipogenic enzyme studied was substantially elevated genetically in LW(f) vs. SW lines (Fig. 8; Zera and Zhao, 2003b). 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, 2003b).

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; Zeta et al., 1997; Zera and Zhao, 2003b, 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
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 observed in SW and (LW[f])-selected lines.

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

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

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, 2003a; D = Zera and Zhao, 2003b; E = A. J. Zera and Z. Zhao unpublished data; F = Zera and Brink, 2000.

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).
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, 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, 2003a, 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, 2003a). 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 [2003b] 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 (Kacer and Burns, 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 (Keterson 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, 2003b). 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, 2003b, 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; \( \text{df} = \text{[number of blocks]} - 1 \)). 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 micro-evolution of metabolism and life histories.

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