Chapter 15
The Biochemical Basis of Life History Adaptation: *Gryllus* Studies Lead the Way

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Abstract During the past two decades, studies of lipid metabolism in the wing-polymorphic cricket, *Gryllus firmus*, have contributed significantly to our understanding of the biochemical mechanisms underlying life history adaptation. Radiotracer studies of *G. firmus* have documented morph differences in the flow of metabolites through pathways of lipid and amino acid metabolism. These differences result in the preferential diversion of nutrients to lipid flight fuel biosynthesis and away from ovarian growth in the flight-capable morph and vice versa in the flightless/high fecundity morph. These studies verify the widely held but previously undocumented hypothesis that trade-offs between life history traits (e.g., dispersal vs. reproduction) seen at the level of the whole organism result from trade-offs in the flow of metabolites through pathways of intermediary metabolism. These differences in pathway flux are produced by global change in the activities of numerous enzymes of lipid metabolism. Genetic, endocrine, and gene expression studies suggest that these changes are due to evolutionary change in regulators that control the activities of whole blocks of enzymes. Morph differences in enzyme activity are caused primarily by altered gene expression leading to altered enzyme concentration without change in kinetic properties. These results bear on long-standing, but as yet unresolved, questions regarding the mechanisms of enzyme microevolution. Finally, ongoing transcriptome profiling has identified additional morph differences in the expression of genes encoding enzymes of glyceride biosynthesis.

Keywords Life history • Trade-off • Wing polymorphism • Evolutionary physiology • Lipid metabolism • Triglyceride biosynthesis

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15.1 Introduction

Life history evolution has been a major focus of research in evolutionary biology since at least the 1960s (Stearns 1992; Roff 2002). Life history traits are key organismal features that determine lifetime patterns of growth, reproduction, longevity, etc. These traits are major components of Darwinian fitness, and variation in suites of life history traits often constitute some of the most important adaptations to different habitats, for different functions, etc. A common feature of life history adaptation is trade-offs or negative associations between various life history traits. For example, in many organisms, reproductive output is negatively associated with dispersal, resulting in phenotypes specialized for dispersal at the expense of reproduction or vice versa.

A long-standing issue in life history evolution has been the physiological basis of life history adaptation (Townsend and Calow 1981; Zera and Harshman 2001; Flatt and Heyland 2011). Physiology is used here in a broad sense to denote any aspect of organismal function (neural, hormonal, metabolic, etc.), studied at any biological level (systemic, biochemical, molecular). Life history traits such as the timing and magnitude of egg production, dispersal, growth, etc. all require various products of metabolic pathways, such as fatty acid fuel for flight or protein for egg yolk production. Thus, evolutionary modifications of flux (i.e., metabolite flow) through pathways of metabolism are expected to be a key aspect of life history adaptation. Moreover, interactions among metabolic pathways, a key feature of intermediary metabolism, provide the functional basis for life history trade-offs.

Until the mid-late 1980s, physiological studies of life history adaptation focused almost exclusively on whole-organism aspects of energetics (respiration rate, concentration of energy reserves) at the level of individual species. Very little was known about specific aspects of intermediary metabolism that were altered to produce life history adaptations. Nor was much known about the biochemical-genetic basis of life history adaptation within species. During the past 15 years, this situation has changed dramatically, and studies involving wing-polymorphic *Gryllus* crickets have led the way in investigations of the biochemical basis of life history adaptation (Zera and Harshman 2001, 2009, 2011).

Lipids play a key role in many life history traits (Townsend and Calow 1981; Zera and Harshman 2001). For example, lipid is an important energy reserve, and many life history adaptations (dispersal and migration; diapause; resistance to starvation) involve enhanced production and storage of lipid (Downer 1985). In addition, individuals exhibiting life history traits, such as increased reproductive output, that trade-off with life history traits mentioned above, often exhibit reduced somatic lipid reserves. Thus, evolutionary modification of lipid metabolism appears to play a pivotal role not only in the evolution of individual life history traits but also with respect to trade-offs between traits (Zera and Harshman 2001, 2011). However, the details of the mechanisms involved, at the level of pathways of metabolism and enzymes involved in these pathways, had not been studied in detail prior to the *Gryllus* work described in this chapter. The present chapter will provide

a brief overview of recent and ongoing *Gryllus* studies with a focus on integrative, multilevel studies of adaptive modification of lipid metabolism. The main goal of these studies has been to understand the chain of causality that runs from adaptive modification of gene sequence/expression leading to modified activities of enzymes and flux (metabolite flow) through whole pathways of intermediary metabolism that ultimately results in changes in whole-organism lipid reserves.

15.2 Background on and Experimental Advantages of Wing Polymorphism in *Gryllus*

Before discussing biochemical studies of lipid metabolism in wing-polymorphic *Gryllus*, a brief description of this polymorphism will be given. Wing polymorphism is common in natural populations of many insect species, having evolved independently in most major insect orders. The polymorphism involves the existence of morphs (discontinuous phenotypes) in the same population that differ in numerous traits, and which are adapted for flight at the expense of egg production and vice versa (see Fig. 15.1). Wing polymorphism is a classic example of a life history trade-off in which the evolution of enhanced allocation to reproductive output is coupled with the evolution of decreased allocation to somatic function (e.g., flight ability). The polymorphism plays a central role in the life cycle of many species (Harrison 1980; Zera and Denno 1997; Guerra 2011; Zera and Brisson 2011).

Like many other wing-polymorphic insects, wing-polymorphic *Gryllus* species contain one morph (LW(f)) that has fully developed wings and flight muscles and is capable of flight but has delayed and reduced egg production relative to the obligately flightless (SW, short-winged) morph that has vestigial wings and underdeveloped flight muscles. A second flightless morph [LW(h)], that is produced from the LW(f) morph via flight muscle histolysis, has many attributes of the SW morph, such as enhanced ovarian growth (Zera et al. 1997). The LW(h) morph is not a major focus of the present chapter (see Zera et al. 1997, and Chap. 7 of this volume). LW(f) and SW morphs in *Gryllus* can be produced due to differences in genetic as well as numerous environmental factors (e.g., temperature, photoperiod, density), as is the case for morphs in most other wing-polymorphic species. However, the primary focus of study in *Gryllus* has been adaptive genetic differences between morphs in physiology, using artificially selected lines that had been derived from field-collected individuals and which were raised in a single environment.

Wing polymorphism in *Gryllus* has many advantages in evolutionary-physiological studies of life history adaptation. Extensive field studies in this and other groups have clearly identified the adaptive basis of the polymorphism in natural populations (Harrison 1980; Roff 1986a; Zera and Denno 1997; Zera et al. 2007a; Guerra 2011). This is not the case for some other life history phenotypes studied in model genetic organisms, particularly laboratory-generated mutants with

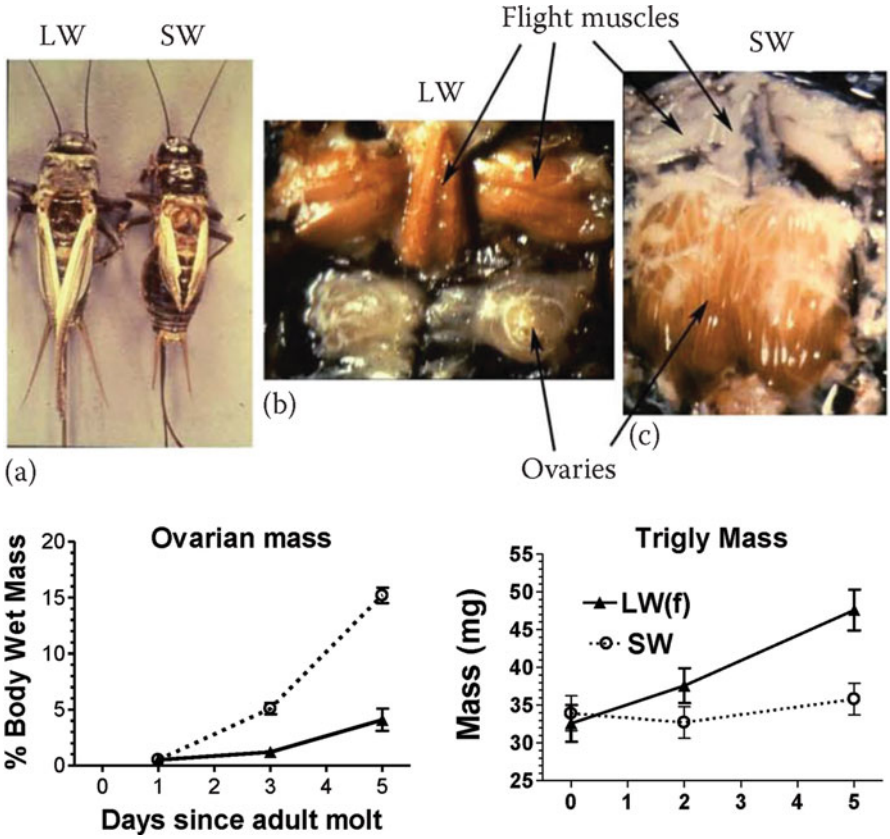

Fig. 15.1 *Top* panel. Day-5 adult female long-winged (LW) and short-winged (SW) morphs of *Gryllus firmus* illustrating (**a**) much larger hind wings and (**b**, **c**) much larger flight muscles, but much smaller ovaries in the LW compared to the SW morph. *Lower* panels. Reduced growth of the ovaries but greater accumulation of whole-body triglyceride in the LW(f) vs. SW morph during the first 5 days of adulthood. In these figures, LW is equivalent to LW(f) (Figure is from Zera 2005)

altered life history traits (e.g., insulin signaling mutants in *Drosophila*). While useful for dissecting the underlying physiological mechanisms of life history trait variation, extrapolating from these mutant studies to evolution in outbred populations is often questionable (Zera and Harshman 2009). This is due to the mutants often exhibiting highly reduced fitness, which is expected to result in their rapid elimination from outbred populations (for additional discussion, see Zera and Harshman 2009, 2011).

Wing-polymorphic crickets are easily reared in the laboratory and are amenable to basic evolutionary-genetic manipulations, such as artificial selection. Individuals of these species are large (ca. 1 g adults), and phenotypic differences between morphs (fecundity, flight muscle mass, enzyme activities) also are very large (see Fig. 15.1). Thus, detailed physiological studies, such as measurement of organ-

specific enzyme activities or in vivo incorporation of radiotracers into reproductive vs. somatic organs, can be routinely undertaken in *Gryllus*. These techniques are much more difficult or are not realistic to employ in much smaller organisms such as fruit flies, aphids, or nematodes (e.g., *C. elegans*). Moreover, the current development of various molecular-genetic techniques (see other chapters in this volume) and "omics" techniques (e.g., transcriptome profiling, proteomics) is reducing some of the technical disadvantages of *Gryllus* compared to model genetic species such as *Drosophila melanogaster*.

In addition to biochemical studies of life history adaptation, *Gryllus* has been used to investigate a variety of other important issues in evolutionary physiology, which cannot be discussed in the present chapter because of space constraints. *Gryllus* work on the endocrine basis of life history adaptation is reviewed in Zera and Harshman (2001, 2009) and Zera (2013) and Chap. 7 of the present volume. Experimental evolution of endocrine regulation per se is reviewed in Zera (2006, 2013) and Zera et al. (2007b). Evolution of hormonal circadian rhythms is covered in Zera and Zhao (2009) and in Chap. 7 of the present volume.

15.3 Studies of Morph-Specific Metabolic Adaptation

15.3.1 Whole-Organism Studies of Nutrient Acquisition and Allocation to Lipid Production

Initial studies of life history adaptation in *Gryllus firmus* and *Gryllus rubens* involved artificial selection on the LW(f) vs. the SW morph in laboratory populations, founded from field-collected individuals, to produce multiple genetic stocks that are nearly pure breeding for the LW(f) or SW morphs (Roff 1986a, b; Roff and Fairbairn 2001, 2007). These early genetic studies quantified the genetic basis of individual traits and negative genetic correlations (i.e., genetic trade-offs) between key traits that comprise the polymorphism: large wings, large flight muscles, and small ovaries vs. small wings, underdeveloped flight muscles, and large ovaries. Documenting the genetic basis of trait variation/covariation is of central importance in evolutionary studies, since trait differences must have a genetic basis in order to evolve. Of particular importance to studies described below, the availability of selected lines provided the material to undertake physiological studies to identify the underlying functional causes of differences in components of flight and reproduction.

Early feeding and whole-organism physiological studies in *Gryllus* provided the background physiological context to investigate the biochemical basis of life history adaptation. These studies were the first to demonstrate experimentally that a life history trade-off within a species was indeed caused by the differential allocation of internal resources to competing life history functions (Mole and Zera 1993; Crnokrak and Roff 2002; Zera and Larsen 2001; reviewed in Zera and

Harshman 2001). LW(f) and SW morphs roughly consumed the same amount of food, but the LW(f) morph allocated a greater proportion of ingested resources to aspects of flight capability (e.g., respiratory maintenance of functional flight muscles and production of lipid flight fuel) at the expense of ovarian growth, while the opposite occurred in the SW morph. Thus the evolution of a flightless SW morph freed up nutrients previously required to construct and maintain various aspects of flight ability (i.e., in the absence of flight), allowing these nutrients to be rechanneled into egg production, resulting in the more fecund SW morph.

In artificially selected lines of *G. firmus*, LW(f) and SW female morphs emerge as adults with low and equivalent triglyceride stores (Fig. 15.1). However, during the first week of adulthood, LW(f) adult females accumulate more somatic triglyceride at the expense of ovarian growth, while the opposite situation occurred in SW adult females. Thus, during the same stage of development, there is a clear trade-off between allocation of nutrients to somatic lipid reserves vs. ovarian growth (Zera and Larsen 2001). Analogous trade-offs between lipid accumulation and egg production have been reported in a number of other species and thus appear to be a widespread physiological characteristic of life history trade-offs (reviewed in Townsend and Calow 1981 and in Zera and Harshman 2001, 2009).

15.3.2 Morph-Specific Modifications of Flux Through Pathways of Intermediary Metabolism

The core of the *Gryllus* research on the metabolic basis of life history adaptation focused on morph-specific alterations in flux through whole pathways of lipid metabolism, activities of enzymes of the pathways, and the causes of enzyme activity differences (e.g., kinetics vs. gene expression) that result in elevated lipid accumulation in the LW(f) morph. Radiotracer studies in the LW(f) and SW genetic stocks demonstrated that flux through the de novo pathway of fatty acid and triglyceride biosynthesis was greater in LW(f) adult females (Zera and Zhao 2003a), while fatty acid oxidation was reduced in LW(f) compared to SW lines (Fig. 15.2). Both of these factors accounted for the greater accumulation of triglyceride flight fuel in the LW(f) morph during early adulthood. This was the first documented example of a trade-off at the level of flux through pathways of intermediary metabolism that underlies a life history trade-off, a widely held, but previously unsubstantiated, assumption in life history research. In addition, in the LW(f) morph, newly biosynthesized triglyceride was preferentially allocated to the fat body, while in the SW morph, newly synthesized lipid was preferentially allocated to the ovaries. Lipid is an important component of eggs as well as an important flight fuel and somatic energy reserve. Thus, in studies of life history trade-offs between reproduction and somatic maintenance, it is important to distinguish between somatic and ovarian lipid. However, this has typically not been done

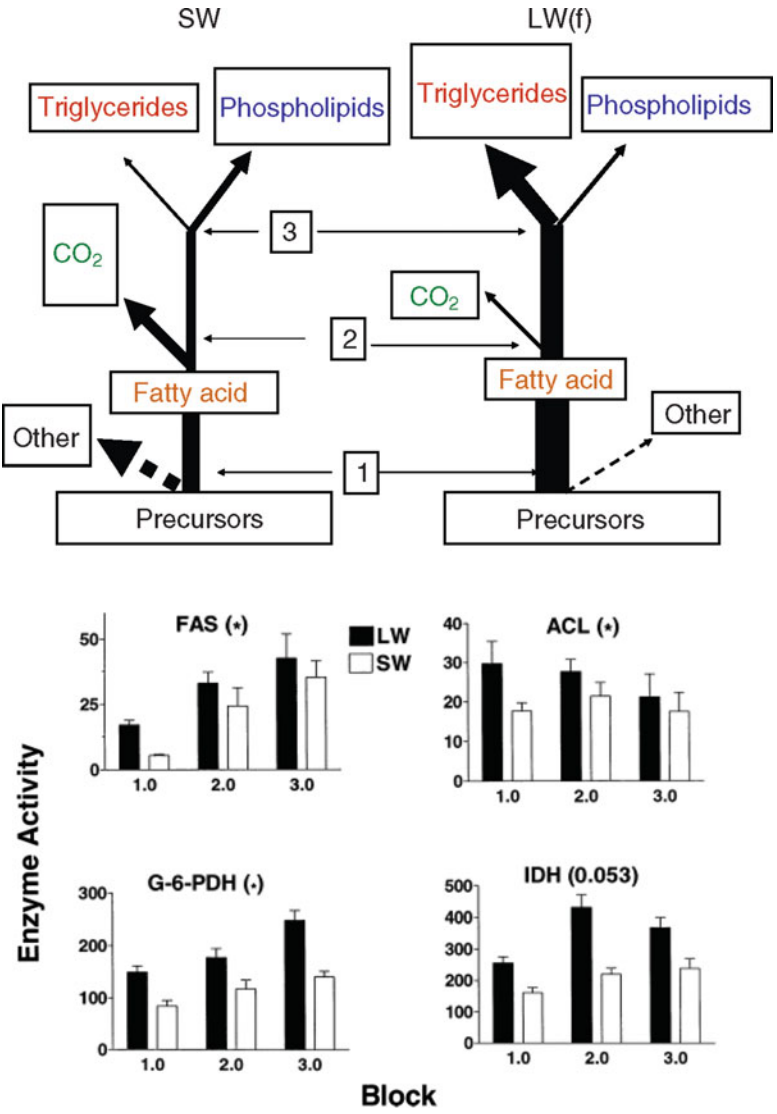

Fig. 15.2 *Top* panel. Results of radiotracer studies illustrating three trade-offs in the biosynthesis or oxidation of lipid classes in the flight-capable (LW(f)) and flightless, reproductive (SW) morphs of *Gryllus firmus*: (*1*) greater incorporation of radiolabel into total lipid vs. other pathways in LW (f), (*2*) greater conversion of fatty acid into triglyceride vs. oxidation to CO_2 in LW(f), and (*3*) greater production of triglyceride vs. phospholipid in LW(f). *Bottom* four panels: higher fat body-specific activities of lipogenic enzymes in three pairs of LW (same as LW(f)) vs. SW artificially selected lines. Block refers to independent selection trial (See Zera 2005). *FAS* fatty acid synthase, *ACL* ATP-citrate lyase, *G-6-PDH* glucose-6-phosphate dehydrogenase, *IDH* $NADP^+$-isocitrate dehydrogenase. *Top* panel is from Zera and Harshman (2011); *bottom* panels are from Zera (2005). Values in *parentheses* refer to the results of paired t-tests comparing LW and SW line means (* = $P < 0.05$)

for small insects such as *Drosophila* and illustrates one of the advantages of the much larger *Gryllus* (Zhao and Zera 2002; Zera 2005).

Another important finding of the *Gryllus* radiotracer studies was the trade-off in production of specific classes of lipids used in particular life history functions. Different classes of lipid can have very different roles. For example, triglyceride is a major energy reserve, while phospholipid is the major structural component of membranes. However, previous life history studies usually did not distinguish between differences in individual classes of lipids between life history phenotypes. Radiotracer studies of lipid biosynthesis mentioned above also identified a trade-off between the production of triglyceride vs. phospholipid: LW(f) females produced more triglyceride at the expense of phospholipid, while the opposite situation occurred in the SW morph (although total lipid biosynthesis is lower in the SW morph) (Fig. 15.2; Table 15.1). The shift to increased production of phospholipid in the SW morph is probably another metabolic adaptation to accommodate increased egg production which requires a greater amount of phospholipid for membranes in developing eggs.

Table 15.1 Examples of morph differences in various biochemical and other traits in adult *Gryllus firmus*

Trait	Morph difference (LW (f) relative to SW)	References
Respiration rate	Higher	A, B, C
Lipid reserves accumulated during adulthood	Higher	D, E
Ovarian growth and egg production	Lower	C, E, F
Rate of total lipid and triglyceride biosynthesis	Higher	E, G, H
Relative rate of triglyceride vs. phospholipid biosynthesis	Higher	E, G
Rate of fatty acid oxidation	Lower	E, I
Rate of amino acid oxidation	Higher	J, K
Conversion of amino acids into ovarian protein	Lower	J, K
Conversion of amino acids into lipid	Higher	J, K
Specific activities of lipogenic enzymes	Higher	E, L
Transcript abundance of lipogenic enzymes	Higher	K, M
Concentration of lipogenic enzymes	Higher	K, M, N
Kinetic properties of purified lipogenic enzymes	Equivalent	K, M, N
Transcript abundance of enzymes of glyceride biosynthesis	Higher	O

LW(f) dispersing morph with long wings and functional flight muscles, *SW* flightless morph with vestigial wings and flight muscles; see Fig. 15.1 and text

References: A = Mole and Zera (1993), B = Crnokrak and Roff (2002), C = Zera and Harshman (2001), D = Zera and Larsen (2001), E = Zera (2005), F = Zera et al. (2007a, b), G = Zhao and Zera (2002), H = Zera and Harshman (2009), I = Zera and Zhao (2003a), J = Zera and Zhao (2006), K = Zera and Harshman (2011), L = Zera and Zhao (2003b), M = Schilder et al. (2011), N = Zera et al. (2014), O = Vellichirammel et al. (2014)

Other large-magnitude differences in flux through pathways of intermediary metabolism were identified between the morphs, most notably, through pathways of amino acid metabolism. For example, compared with the LW(f) females, SW females converted a greater amount of amino acids into egg protein, oxidized a smaller proportion of amino acids, and converted a smaller amount of amino acids into somatic lipid reserves (Zera and Zhao 2006; reviewed in Zera and Harshman 2009; Table 15.1). There results collectively show that specialization for flight vs. reproduction has evolved by extensive remodeling of metabolite flow through various pathways of intermediary metabolism.

15.3.3 Enzyme Activities

Subsequent studies documented large-magnitude differences between the morphs in activities of numerous lipogenic enzymes that give rise to the elevated flux through the lipogenic pathway in the LW(f) morph (Zera and Zhao 2003b; Fig. 15.2). A similar positive association was observed between enzyme activity and life history traits and/or triglyceride concentration in *Drosophila melanogaster* (Luckinbill et al. 1990; Harshman and Schmidt 1998; Merritt et al. 2006; reviewed in Zera and Harshman 2009, 2011). The emerging picture from these biochemical studies is the global evolutionary modification of the activities of many, possibly most, enzymes involved in intermediate metabolism between the morphs of *G. firmus*. This likely has occurred via evolutionary changes in as yet unidentified metabolic regulators that coordinately control the activities of blocks of enzymes in whole pathways of metabolism (Zera and Zhao 2003b; Zera 2011), as has occurred in laboratory evolution of glucose metabolism in yeast (Ferea et al. 1999).

This hypothesis is supported by the essentially perfect co-segregation of physiological, morphological, and reproductive traits in adult F_2 female morphs produced by interstock crosses between LW(f) and SW lines and backcrosses (Zera and Zhao 2003a, b). Thus, F_2 LW(f) females had higher lipogenic enzyme activities, higher flux through the lipogenic pathway, reduced fatty acid oxidation, larger wings and flight muscles and smaller ovaries compared with F_2 SW females. Differences in these traits between F_2 LW(f) and SW morphs were of the same magnitude as differences between LW(f) and SW female parents used to produce the F_1 generation. This very strong co-segregation is inconsistent with the various morphological, reproductive, and biochemical features being inherited independently of each other. Rather, their coordinate expression appears to be most reasonably explained by a master polymorphic regulator or set of regulators (hormonal or other regulators) with pleiotropic effects on these various traits (Zera and Zhao 2003b). Hormone manipulation experiments also support this hypothesis: application of a juvenile hormone analogue to LW(f) female adults changed each trait mentioned above (except for wing length which cannot be modified in adults) to values seen in SW, unmanipulated females (Zera and Zhao 2004). A major question for future research is the nature of the regulator(s) that

control the suite of variable traits that differ between the morphs. This intensive study of modification of intermediary metabolism in the context of life history adaptation remains one of the most important contributions of *Gryllus* to our knowledge of the physiological mechanisms underlying life history adaptation (Zera 2005, 2009; Zera and Harshman 2009, 2011). There are currently no comparable studies of other life history adaptations that occur in natural populations.

15.3.4 Enzyme Kinetics and Expression

Ongoing research is identifying the specific biochemical and molecular causes of increased activity of lipogenic enzymes that underlie the increased triglyceride production of the LW(f) morph (Schilder et al. 2011; Zera et al. 2014; Table 15.1). These studies are focusing on three enzymes, each of which plays an important role in lipogenesis: $NADP^+$-isocitrate dehydrogenase ($NADP^+$-IDH), an important producer of NADPH required for de novo fatty acid biosynthesis; 6-phosphogluconate dehydrogenase (6-PGDH), a pentose-shunt enzyme which is another important producer of NADPH; and ATP-citrate lyase (ACL), an enzyme in the de novo pathway of fatty acid biosynthesis.

Investigations of $NADP^+$-IDH, the most extensively studied of the three enzymes to date, documented no significant differences in kinetic constants (e.g., substrate or cofactor Michaelis constants (K_M) or turnover number (k_{cat})) between LW(f) and SW enzymes (Schilder et al. 2011). Nor were any non-synonymous DNA sequence differences (with one exception) observed between the coding region of the $NADP^+$-IDH gene in multiple sequences from each of three LW (f) vs. three SW lines. Thus, with one exception in one of 24 sequences, amino acid sequences were identical in $NADP^+$-IDHs in all LW(f) and SW lines. Most of the difference in fat body $NADP^+$-IDH activity between the morphs was due to the difference in transcript abundance which leads to a corresponding difference between the morphs in enzyme concentration (Fig. 15.3, Table 15.1). A similar situation was observed for the pentose-shunt enzyme 6-phosphogluconate dehydrogenase, another important producer of NADPH (Zera et al. 2014). Like $NADP^+$-IDH, higher 6-PGDH enzyme activity in LW(f) female fat body was due to elevated enzyme concentration, with no significant differences in kinetic properties of the enzyme from LW(f) vs. SW lines. In this case, however, elevated enzyme concentration in LW(f) fat body was primarily due to greater enzyme stability and, possibly, elevated gene expression (Zera unpublished). The third enzyme, ACL, also exhibits higher transcript abundance in the fat body of LW(f)-selected lines and no kinetic differences between the enzyme from LW(f) and SW lines. Enzyme concentration has yet to be measured for this enzyme.

These studies not only represent the most intensive investigations of the enzymological differences underlying life history adaptation in an outbred population, they also represent one of the handful of detailed investigations of intraspecific enzyme adaptation. Specifically, studies of $NADP^+$-IDH and 6-PGDH in *Gryllus*

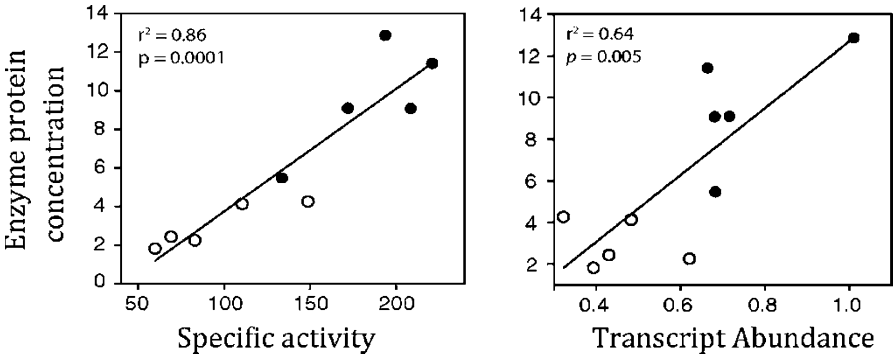

Fig. 15.3 Associations between enzyme protein concentration and specific activity or relative transcript abundance for $NADP^+$-isocitrate dehydrogenase in individuals from one pair of LW (f) (*filled circles*) or SW (*open circles*) selected lines of *G. firmus*. Note the strong covariation between enzyme activity and enzyme protein concentration and enzyme concentration and transcript abundance (Data from Schilder et al. 2011)

have identified the relative importance of evolutionary change in enzyme concentration vs. kinetic properties of the enzyme to adaptive difference in enzyme activity, a central topic of study in population genetics since the 1970s (Storz and Zera 2011; Schilder et al. 2011). $NADP^+$-IDH investigations in *Gryllus* also are among the few that provide information on the functional significance of variation in transcript abundance and the causal connection between variation in protein function and changes in flux through pathways of metabolism.

15.3.5 Morph-Specific Transcriptome Profiling

Previous work on the biochemical basis of life history adaptation and trade-offs in *G. firmus*, discussed above, focused on candidate enzymes and pathways of lipid and amino acid metabolism. Ongoing transcriptome profiling using RNA-Seq is focusing on morph-specific global changes in gene regulation, measured in the fat body (Vellichirammel et al. 2014, unpublished data). Only a few results of that study will be mentioned here. First, the LW(f) morph exhibited elevated transcript levels compared to the SW morph for a number of enzymes of glyceride biosynthesis, such as 1-acylglycerol-3-phosphate acyltransferase, phosphatidate phosphatase, and glycerol-3-phosphate dehydrogenase. These data are consistent with the rate of triglyceride biosynthesis being elevated in the LW(f) compared to the SW morph, mentioned above. Indeed, differences in transcript abundance for these enzymes are much greater in magnitude (e.g., greater than tenfold for 1-acylglycerol-3-phosphate) than morph differences in either transcript abundance (less than twofold) or specific activity (twofold, Fig. 15.2) for enzymes of the de novo fatty acid biosynthetic pathway. This suggests that the elevated rate of

triglyceride biosynthesis in the LW(f) morph might be primarily due to upregulation of the glyceride portion of lipogenesis (in which fatty acids are linked to glycerol phosphate) and less to the biosynthesis of fatty acids, which has been the main focus of previous enzyme studies of lipid biosynthesis. These transcriptome data provide additional tools to investigate molecular aspects of morph-specific triglyceride biosynthesis in *G. firmus*.

Second, the fat body of the reproductive SW morph exhibited a substantially (tenfold) elevated level of an insulin-related peptide transcript compared to the LW (f) morph. Insulin-related peptides are thought to be important regulators of reproduction, metabolism, and trade-offs between these traits in a variety of insects (Zera and Harshman 2009; Zera et al. 2007b; Flatt and Heyland 2011). The homologue of the transcript elevated in SW *Gryllus* also is substantially elevated in the gregarious phase of the desert locust that exhibits earlier sexual maturation and egg production compared with the solitary phase (Badisco et al. 2008). Identification of this important endocrine regulator opens up the possibility of investigating insulin-like peptide regulation of morph-specific reproduction and metabolism in *Gryllus*.

15.4 Summary, Conclusions, and Future Directions

Over several decades, *Gryllus* crickets have made important contributions to the nascent field of evolutionary physiology and continue to be at the forefront of several areas of research in this field (Zera and Harshman 2001, 2009, 2011). Detailed studies of morph-specific differences in lipid metabolism in *G. firmus* currently constitute the most detailed analysis of evolutionary modification of intermediary metabolism in the context of life history adaptation in outbred populations. In particular, identification of trade-offs in flux through several pathways of intermediary metabolism (Zhao and Zera 2002; Zera and Zhao 2006; Zera and Harshman 2011) provided that first direct confirmation of the widespread assumption in life history physiology that life history trade-offs at the level of whole organisms result from trade-offs in the flow of metabolites through pathways of metabolism. These studies set the stage for future investigations of the regulators that control the differential flux through interacting pathways in morphs. Moreover, enzymological-genetic studies of lipogenic enzymes from LW(f) and SW morphs (Schilder et al. 2011; Zera et al. 2014) constitute one of the most detailed studies of intraspecific enzyme adaptation. This was a prominent area of research in population genetics, but has languished since the 1980s as the field moved to statistical analyses of DNA sequence variation of enzymes to identify the role of natural selection in enzyme microevolution. The recent *Gryllus* studies may help to spur a renewal in functional investigations of enzyme microevolution. Finally, transcriptome studies are identifying global changes in gene expression that contribute to adaptive differences between wing morphs (Vellichirammel et al. 2014, unpublished data). Some data, such as the greater expression of genes encoding enzymes of the triglyceride pathway in the LW(f) morph, verify previous

radiotracer studies of pathway flux. Important new findings are also emerging from these studies, such as morph-specific differences in components of the insulin signaling. The transcriptome study may also be important for identifying morph-specific differences in regulators responsible for the differences in metabolism between morphs. Finally, the ongoing development of various molecular tools, discussed in various chapters of this volume, will also be invaluable with respect to conducting more sophisticated experiments that test various ideas regarding endocrine and biochemical aspects of morph adaptation discussed in this chapter.

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