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Intermediary Metabolism and Life-History Trade-Offs: Differential Metabolism of Amino Acids Underlies the Dispersal-Reproduction Trade-Off in a Wing-Polymorphic Cricket

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Submitted March 31, 2005; Accepted January 5, 2006;
Electronically published April 11, 2006

ABSTRACT: Although the differential flow of metabolites through alternate pathways of intermediary metabolism is thought to be an important functional cause of life-history trade-offs, this phenomenon remains understudied. Using a radiolabeled amino acid, we quantified genetic differences in *in vivo* amino acid metabolism between morphs of the wing-polymorphic cricket *Gryllus firmus* that trade off early-age reproduction and dispersal capability. Lines selected for the flight-capable morph, which delays reproduction, oxidized a greater proportion of radiolabeled glycine and converted a greater amount into somatic lipid, mainly triglyceride (flight fuel). By contrast, lines selected for the flightless, reproductive morph converted a substantially greater proportion of glycine into ovarian protein. Compensatory interactions between amino acid and lipid metabolism make up a key aspect of specialization for dispersal versus reproduction in *G. firmus*: increased oxidation of amino acids by the flight-capable morph spares fatty acid for enhanced conversion into triglyceride flight fuel. By contrast, increased oxidation of fatty acid by the flightless morph spares amino acids for enhanced biosynthesis of ovarian protein. Studies of amino acid and lipid metabolism in *G. firmus* currently represent the most detailed analyses of genetic modifications of intermediary metabolism that underlie a functionally important life-history trade-off found in natural populations.

Keywords: life history, trade-off, wing polymorphism, *Gryllus*, intermediary metabolism, allocation.

Trade-offs between life-history traits such as reproduction, longevity, and dispersal have been a central focus of life-history research for decades (Stearns 1992; Zera and

Harshman 2001; Dufty et al. 2002; Ricklefs and Wikelski 2002; Roff 2002). Numerous studies have attempted to identify the functional causes of trade-offs, with most work focusing on the differential allocation of internal resources to competing life-history traits (Tinkle and Hadley 1975; Pianka 1981; Boggs 1997a; Rose and Bradley 1998; Zera and Harshman 2001; Zhao and Zera 2002). However, *in vivo* mechanisms of differential allocation remain poorly understood at the physiological level, while biochemical investigations of this phenomenon are just beginning (Boggs 1997a; Zera and Harshman 2001; O'Brien et al. 2002, 2005; Zhao and Zera 2002).

Trade-offs in internal resource allocation must result in large part from the differential flow of metabolites through pathways of intermediary metabolism. However, few data exist on the mechanisms involved. This is especially the case for genetically based life-history trade-offs, which are particularly relevant for understanding the mechanisms of life-history evolution. For example, only a few studies have documented *in vitro* differences in activities of enzymes of intermediary metabolism between lines that have been selected for different life histories (Harshman and Schmidt 1998; Harshman et al. 1999; Zera and Zhao 2003a). Only one study has reported variation in *in vivo* flux through a pathway of intermediary metabolism (de novo biosynthesis of triglyceride and phospholipid) that appears to contribute to a genetically based life-history trade-off (Zhao and Zera 2002). In short, the biochemical-metabolic mechanisms underlying life-history variation and evolution largely remain a black box.

Alterations in amino acid metabolism are expected to play an especially important role in the adaptive divergence of life histories. Amino acids can be metabolized by a variety of pathways (Clements 1959; Candy 1985; Stryer 1988), whose products can contribute significantly to somatic or reproductive functions (Boggs 1992). Amino acids are precursors of somatic (e.g., flight muscle) or re-

productive proteins (e.g., vitellogenin; pathway 1 in fig. 1A); they can also serve as an important energy source via conversion to Krebs cycle intermediates (pathway 2 in fig. 1A) and subsequent oxidation to CO₂ (Clements 1959; pathway 3 in fig. 1A); finally, amino acids can be transformed, via the Krebs cycle, into a variety of somatic or reproductive macromolecules, such as lipid (Kon and Monroe 1971; pathway 4 in fig. 1A). The evolution of new life histories is expected to require changes in the relative flux through these pathways, for example, when relative somatic versus reproductive effort is altered during the course of adaptation. Although variation in amino acid acquisition from the diet has been investigated in ecological studies of interspecific life-history trade-offs (Boggs

1997*a*, 1997*b*; O'Brien et al. 2002, 2005), to our knowledge, no study has investigated genetic modifications in amino acid metabolism that contribute to within-species variation in life-history specialization.

Wing polymorphism in insects is a powerful experimental model that has been used extensively to investigate the physiological, and more recently, the biochemical basis of life-history evolution (Zera and Denno 1997; Zera et al. 1997, 1998; Zera and Harshman 2001; Zhao and Zera 2002; Zera and Zhao 2003*a*, 2004). *Gryllus firmus*, the most intensively studied species in this regard, exists in natural populations as two genetically specified morphs. One morph, adapted for flight at the expense of reproduction, has functional (long) wings and functional (large, pink)

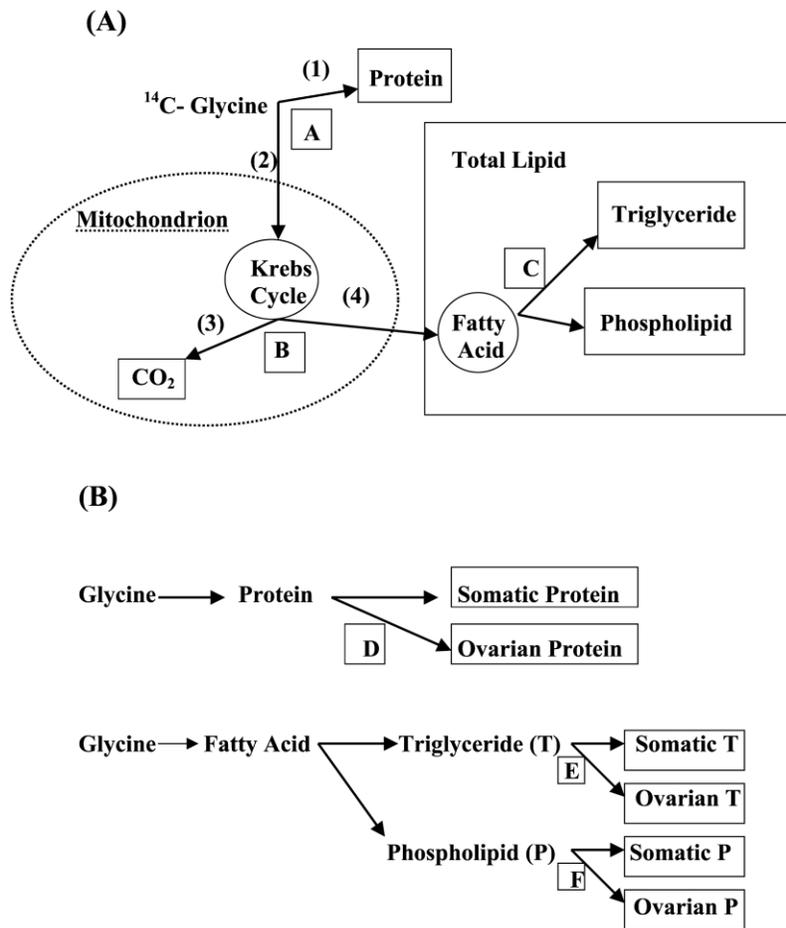


Figure 1: A, Metabolic fates of the amino acid glycine, investigated in this study: utilization for protein biosynthesis (pathway 1), oxidation to CO₂ via the Krebs cycle (pathways 2 and 3), and transformation into lipid (pathways 2 and 4). Other metabolic fates are possible (e.g., conversion into carbohydrate or diversion through other pathways in the Krebs cycle) but were not investigated. Metabolites (rectangles) were quantified, while intermediates (circles) were not. A–C denote the three metabolic trade-offs investigated. Values (proportional diversion of radiolabel) for these trade-offs are given in table 1. B, Allocation trade-offs (proportional diversion of end products of metabolism to ovaries vs. soma [whole body minus ovaries]). Values for these trade-offs are given in table 1.

flight muscles (denoted LW(f)). The alternate, obligately flightless morph has reduced wings and vestigial flight muscles (denoted SW; Zera et al. 1997; Zera and Harshman 2001). During the first week of adulthood, the LW(f) morph biosynthesizes a much larger quantity of somatic triglyceride, the main flight fuel of *Gryllus* (Zera et al. 1999; Zera and Larsen 2001), but it exhibits ovarian growth that is only 25%–50% that of its SW counterpart. Thus, during the first week of adulthood, LW(f) and SW morphs differentially allocate (i.e., trade off) internal nutrients into reproduction versus the soma (i.e., flight capability).

During the past few years, biochemical studies of the trade-off between flight capability and early-age reproduction in *G. firmus* have largely focused on morph-specific alterations of in vivo lipid metabolism (Zhao and Zera 2002; Zera and Zhao 2003a, 2003b, 2004; Zera 2005). For reasons discussed above, alterations in amino acid metabolism are also expected to play a pivotal role in adaptations for flight versus reproduction, both independent of and in concert with changes in lipid metabolism. In this study, we used a radiolabeled amino acid to quantify genetically based trade-offs in whole-organism amino acid metabolism and allocation to somatic versus reproductive organs in lines of *G. firmus* artificially selected for LW(f) versus SW morphs. Moreover, we identified several striking morph-specific interactions between amino acid and lipid metabolism. These metabolic and allocation trade-offs appear to contribute significantly to genetic specializations for dispersal versus early age reproduction, a key life-history trade-off.

Material and Methods

Background on Gryllus firmus: Morphs, Selected Lines, and Rearing Conditions

Gryllus firmus, the sand field cricket, occurs in natural populations in the southeastern United States as a long-winged morph, some of which are capable of flight, or as a short-winged morph that is obligatorily flightless (Veazy et al. 1976; Zera et al. 1997). Except in a few rare cases, all short-winged females molt into adults with white, non-functional, underdeveloped flight muscles. All long-winged females initially have fully developed (pink) flight muscles at or shortly after the adult molt (denoted LW(f)); however, flight muscles degenerate in some of these females, rendering them flightless. In artificially selected lines used in this study, LW(f) females typically (>95%) retained their flight muscles by the end of the first week of adulthood, when biochemical studies were performed. Thus, the long-winged, flightless morph is not considered here (see Zera et al. 1997 and Zhao and Zera 2001 for physiological and biochemical information on this morph).

Glycine metabolism was measured in females from three pairs (blocks) of LW(f)- or SW-selected lines during the twenty-third generation of selection. All lines had been derived from the same base population, which, in turn, had been derived from crickets collected in Gainesville, Florida (Zera and Cisper 2001). All lines had been raised under the same temperature ($28^{\circ} \pm 1^{\circ}\text{C}$) and photoperiod (16L : 8D) and had been fed the standard (100%) diet since their inception (see Zera and Cisper 2001 and Zera 2005 for details of artificial selection). Adult emergence occurred sequentially in the three pairs (blocks) of LW(f) and SW lines, which allowed characterization of LW(f) and SW lines from one block before emergence of adults from the other blocks. Lines were close to being pure breeding (i.e., producing >95% of the selected morph) when experiments were conducted, and they were the same lines used in previous studies of lipid metabolism and life-history evolution (Zera and Bottsford 2001; Zera and Cisper 2001; Zhao and Zera 2002; Zera and Zhao 2003a; see "Discussion"). In this study, mean wet weight did not differ between LW(f) and SW lines on day 5 of adulthood (paired *t*-test of line means: $t = 2.51$, $df = 2$, $P = .13$).

Incorporation of ^{14}C -Glycine into Lipid Classes, CO_2 , and Protein

Radiotracer studies were conducted on day 5 of adulthood because this is the period of adulthood when there is a dramatic morph-specific trade-off between ovarian growth and the accumulation of triglyceride (Zera and Larsen 2001). Two μL of sterile saline (0.9% NaCl) containing 8.8×10^5 disintegrations per minute (DPM) of [^{14}C]glycine (4.2 GBq/mmol; 112.7 mCi/mmol) were injected into the abdominal hemocoel of adult female crickets that had been starved for 4 h. Crickets were incubated individually in sealed 50-mL flasks that contained a cup of aqueous NaOH near the top of the flask to trap respired CO_2 . Crickets were incubated for 5 h, which was within the period of time during which incorporation of radiolabel from glycine into CO_2 , whole-body protein, and total lipid was linear (r^2 values from linear regressions ranged from 0.98 to 0.80; $P = .001$ –.04). Thus, the basic data obtained in this study were rates of conversion of carbon from glycine into various end products of metabolism. After the incubation period, radiolabel incorporated into respired CO_2 was quantified as described previously (Zera and Zhao 2003b). For reasons discussed below, radiolabeled products were quantified separately in the ovaries and soma (whole body minus ovaries). Ovaries were removed from crickets, and ovaries and soma were separately homogenized in chloroform/methanol (2 : 1) to extract total lipid. Radioactivity in an aliquot of the total-lipid extract was quantified by liquid scintillation counting as de-

scribed earlier (Zhao and Zera 2002). Somatic lipid and ovarian lipid were further separated into their main components, triglyceride and phospholipid (which make up >90% of total lipid; Zera and Larsen 2001), by thin-layer chromatography of an aliquot of the total-lipid extract (Zhao and Zera 2002). Radioactivity in triglyceride and phospholipid was quantified as described previously (Zhao and Zera 2002). Amount of radioactivity incorporated into total protein was measured essentially as described by Geer and Downing (1972). The fat-free cricket residue was washed twice with 10% trichloroacetic acid, which removed unincorporated ^{14}C -glycine and radiolabel from glycine incorporated into soluble carbohydrate and other amino acids. The protein residue contained no detectable carbohydrate, as determined by the anthrone assay (Zera and Larsen 2001), and was solubilized by addition of 0.5 mL of 60% perchloric acid and 1 mL of hydrogen peroxide, followed by incubation at 70°–80°C for 1 h. Incorporation of glycine into carbohydrate was not measured because carbohydrate levels are low in morphs of *G. firmus* and do not appear to contribute significantly to the trade-off between early-age reproduction and flight capability (Zera and Larsen 2001).

Experimental Strategy and Rationale

This study consisted of two main parts, each of which had two components. In the first part, we tested whether LW(f) and SW morphs differ genetically in whole-organism flux (rate) through individual pathways of amino acid metabolism and in allocation of end products of metabolism to individual body compartments (amount of metabolic products accumulated in ovaries or soma [body minus ovaries] during the assay period). In the second part, we investigated morph-specific genetic trade-offs in proportional flux through bifurcating metabolic pathways (metabolic trade-offs) and in proportional allocation of metabolic products to the soma versus ovaries (allocation trade-offs), using data obtained in part 1. We quantified morph differences in flux through metabolic pathways and in allocation of end products to somatic versus reproductive body compartments because both of these factors are key biochemical components of life-history adaptations. For example, amino acids can be transformed into triglyceride, which can function as either a somatic (flight fuel) or a reproductive energy reserve, depending on whether biosynthesized triglyceride is transported to somatic or reproductive body compartments.

In the first part of the study, we quantified the amount of radiolabeled carbon from injected ^{14}C -glycine that was incorporated into protein (utilization of amino acid for protein biosynthesis; pathway 1 in fig. 1A), CO_2 (amino acid oxidation for energy production; pathways 2 and 3

in fig. 1A), and lipid (conversion of amino acid into total lipid and various functionally important lipid components; pathways 2 and 4 in fig. 1A). We first tested for morph-specific differences in whole-organism rates of production of individual metabolic products (e.g., protein or lipid) from glycine by pooling metabolite data from ovaries and soma (statistical analysis described below). We then tested for differences between the morphs in compartment-specific allocation of end products by comparing the morphs with respect to the quantity of various end products separately in ovaries or soma.

Although amino acids can be converted to a variety of important macromolecules (e.g., lipid, carbohydrate), lipid was the focus of conversion of amino acid into a non-protein constituent. This was done because a large proportion of amino acid can be converted into lipid (see the introduction to this article), and lipid is composed of several key constituents (triglyceride and phospholipid), each of which can have important somatic or reproductive functions. Large-magnitude differences in lipid metabolism have previously been documented between morphs of *G. firmus* (Zhao and Zera 2002; Zera 2005).

The focus of the second part of the study was morph-specific metabolic and allocation trade-offs. Metabolic trade-offs (denoted by capital letters in fig. 1A) are defined as morph differences in the proportional, whole-organism production of a pair of metabolites. Allocation trade-offs (denoted by capital letters in fig. 1B), by contrast, are defined as the proportions of a particular end product of metabolism that are found in the somatic versus the ovarian body compartments. For example, we investigated whether morphs differed in the proportion of glycine that was used for protein biosynthesis versus that converted through the Krebs cycle into nonprotein constituents (CO_2 plus lipid; trade-off A in fig. 1A) or the proportional allocation of biosynthesized protein to somatic versus ovarian body compartments (trade-off D in fig. 1B). It is important to note that the extent to which morphs differ in flux through a single pathway or allocation to a single body compartment is independent of whether they differ in proportional metabolism through linked pathways or proportional allocation to different body compartments. Statistical analyses of these trade-offs are discussed below, and table 1 describes calculation of proportions.

Estimating various trade-offs and testing for their statistical significance required pooling data on radiolabel incorporation in various ways, which were determined by whether metabolic or allocation trade-offs were being tested and by specific aspects of the pathways being compared (see fig. 1A). Because metabolic trade-offs were defined in terms of whole-organism production of metabolites, these trade-offs always involved pooling data from somatic and ovarian body compartments to obtain whole-

Table 1: Proportional diversion (trade-off) of radiolabel from ^{14}C -glycine to various metabolites in whole bodies and various body compartments in flight-capable (LW(f)) or flightless (SW) *Gryllus firmus*

Trade-off ^a	Mean proportional diversion of radiolabel ^b						P value for genetic tests of morph-specific trade-offs ^c
	LW(f)			SW			
	Block 1	Block 2	Block 3	Block 1	Block 2	Block 3	
Metabolic trade-offs:							
(A) Conversion into nonprotein/(protein + nonprotein)	.34 ± .01	.22 ± .03	.15 ± .02	.26 ± .02	.14 ± .02	.09 ± .01	<.01
(B) Conversion into CO ₂ /(CO ₂ + lipid)	.82 ± .02	.74 ± .03	.70 ± .03	.83 ± .03	.74 ± .03	.44 ± .04	>.05
(C) Conversion into phospholipid/(phospholipid + triglyceride)	.41 ± .02	.48 ± .02	.52 ± .03	.53 ± .02	.54 ± .01	.63 ± .02	<.05
Allocation trade-offs:							
(D) Allocation of biosynthesized protein to ovaries/(ovaries + soma)	.14 ± .02	.12 ± .01	.22 ± .02	.31 ± .01	.32 ± .02	.34 ± .0	<.025
(E) Allocation of biosynthesized triglyceride to ovaries/(ovaries + soma)	.10 ± .01	.14 ± .02	.14 ± .02	.29 ± .02	.54 ± .03	.50 ± .01	<.05
(F) Allocation of biosynthesized phospholipid to ovaries/(ovaries + soma)	.10 ± .02	.11 ± .02	.13 ± .02	.19 ± .02	.33 ± .04	.38 ± .03	.06

Note: Metabolic trade-offs describe whole-body, proportional diversion of radiolabeled carbon from glycine through alternate metabolic pathways; allocation trade-offs describe differential allocation of biosynthesized compounds to soma versus ovaries. Proportions were arcsine-transformed before analyses; see “Material and Methods” for rationale and additional information on analyses.

^a Letters refer to trade-offs illustrated in figure 1.

^b Disintegrations per minute (DPM) in right arm/(DPM in right and left arms) of branch points illustrated in figure 1; values are means (± SEM) of selected lines based on six to nine individuals per line. “Right arm of branch point” is defined as the right arm when the branch point is oriented such that flux (direction of the arrow) is facing upward (e.g., pathway 2 of branch point A). Note that the same results are obtained in statistical analyses irrespective of which arm of the trade-off is chosen as the numerator.

^c Results of paired *t*-tests of mean proportional divergence between artificially selected LW(f) and SW line means.

organism values. By contrast, allocation trade-offs, which were defined as the proportional diversions of a single metabolic product to different body compartments, never involved data pooling. Other types of pooling were required to test specific trade-off hypotheses. For example, the first trade-off tested was the proportional diversion of carbon from glycine into protein versus into nonprotein metabolites (i.e., carbon derived from glycine metabolized through the Krebs cycle to produce CO₂ and lipid; trade-off A in Fig. 1A). To test for the existence of trade-off A, we compared radiolabeled whole-body (somatic + ovarian) protein and nonprotein metabolites (CO₂ + total lipid in ovaries and soma) in LW(f) and SW morphs (see table 1 for calculation of proportions). The next trade-off (trade-off B in fig. 1) involved the morph-specific proportional diversion of radiolabel from glycine into CO₂ versus total (somatic + ovarian) lipid (i.e., the relative oxidation of glycine for energy production vs. conversion into all lipid components). The final trade-off tested consisted of morph-specific, proportional diversion of radiolabel from glycine into total (whole-body) triglyceride (energy storage) versus total phospholipid (structural lipid). Three morph-specific, organ allocation trade-offs involving the proportional incorporation of protein, triglyceride, or phospholipid biosynthesized from glycine into ovarian versus somatic body compartments were tested. These allocation trade-offs are illustrated in figure 1B, and calculation of proportions is described in table 1.

Each test of a morph-specific difference in flux through an individual pathway of metabolism or allocation of an individual product of metabolism to a somatic or ovarian compartment was independent of the other tests. The same was true for all tests of metabolic and allocation trade-offs. Furthermore, all tests were planned a priori contrasts used to investigate specific metabolic or allocation hypotheses. Thus, *P* values from individual tests do not require Bonferroni corrections.

Statistical Analyses of Genetic Differences between LW(f) and SW Morphs in Biochemical Traits

Genetic differences between LW(f) and SW morphs with respect to glycine metabolism are particularly relevant for evolutionary-biochemical studies of life histories and were the main focus of this study. Determining whether morphs differ genetically in various biochemical traits is tantamount to determining whether lines directly selected for morph (LW(f) or SW) exhibit significant indirect (correlated) responses to selection on the biochemical traits in question. We used paired *t*-tests to determine whether grand means of a particular biochemical trait (mean incorporation into a particular metabolic product or mean proportional incorporation) differed between the three

pairs of LW(f)- and SW-selected lines. That is, the six line means of a biochemical trait were treated as six individual data points in the *t*-test, similar to analyses conducted by Rose et al. (1996), Zera and Cisneros (2001), Zhao and Zera (2002), and Zera and Zhao (2003a, 2003b). Because artificial selection was conducted in three blocks, each containing a LW(f) and a SW line, line means were compared via paired *t*-tests (as in the studies mentioned above). In these paired *t*-tests, degrees of freedom are equal to 1 minus the number of blocks (i.e., *df* = 2 in all tests), and thus, these tests were very conservative, detecting only strong genetic differences in amino acid metabolism between LW(f)- and SW-selected lines.

Line means of radioactivity incorporated into a metabolic product compared in the paired *t*-tests were adjusted least squares means of the three LW(f)- and SW-selected lines, estimated from ANCOVAs. The independent variable in the ANCOVAs was DPM (radioactivity) of the metabolic product in whole bodies or in the body compartment in question, while the covariate was total DPM from glycine incorporated into all metabolites in all body compartments (total incorporated counts [TC]). These ANCOVAs thus standardized incorporation of radiolabel into a particular metabolite to total incorporation, which eliminates the possibility that a difference between morphs in incorporation of radiolabel into a specific metabolite is simply due to a difference in TC. The *t*-tests of mean proportional incorporation were conducted on arcsine-transformed mean proportions of LW(f) and SW lines estimated from ANOVAs.

Results

Ovaries from SW lines were four to five times heavier than those of LW(f) lines on day 5 of adulthood (mean ovarian mass as percentage of whole-body wet mass for the three SW lines: 21.2% ± 3.3% [*n* = 7], 19.4% ± 1.7% [*n* = 8], and 42.9% ± 2.7% [*n* = 7]; corresponding ovarian masses for the three LW(f) lines: 4.1% ± 3.3% [*n* = 7], 5.2% ± 1.6% [*n* = 9], and 7.6% ± 3.2% [*n* = 5]; *P* < .025; paired *t*-test of mean ovarian mass standardized to whole-body wet mass by ANCOVA). Conversely, all LW(f) females had pink (functional) flight muscles that were larger than the white (nonfunctional) flight muscles of SW females (visual inspection). Thus, in this study, the standard trade-off between ovarian mass and flight capability (wing size and flight-muscle mass/functionality) was observed between LW(f) and SW females.

Significant genetic differences, assessed by paired *t*-tests, were observed between LW(f)- and SW-selected lines, with respect to rate of metabolism of glycine through each of the three pathways studied and in each case of allocation of an end product of metabolism to a particular body

compartment (figs. 2, 3). Documentation of genetic differences between morphs in metabolic flux and allocation, despite the low statistical power of the *t*-tests (i.e., *df* = 2 in each test), implies that these genetic differences are of large magnitude.

The majority of radiolabel from ^{14}C -glycine was incorporated into protein (range of mean incorporation for the six LW(f) and SW lines: 66%–91%), followed by oxidation to CO_2 (9%–28%) and conversion into lipid (4%–6%; figs. 2, 3). In general, LW(f) lines transformed a greater amount of glycine into nonprotein products during the assay period and allocated a greater amount of end products into somatic tissues than did SW lines. For example, compared with SW lines, LW(f) lines diverted approximately 9% less

radiolabel into protein and 33% more radiolabel into CO_2 , and they allocated 13% more newly biosynthesized protein and 68% more newly biosynthesized lipid into the soma versus ovaries (figs. 2, 3).

The above results demonstrate large-magnitude genetic differences between morphs with respect to the rate of metabolism of glycine through individual pathways and allocation of end products of metabolism to individual body compartments. The patterns of morph-specific differences in these traits, such as lower incorporation of radiolabel from glycine into protein coupled with greater incorporation into lipid or CO_2 in LW(f) versus SW morphs, suggest but do not definitively document the existence of morph-specific metabolic and allocation trade-

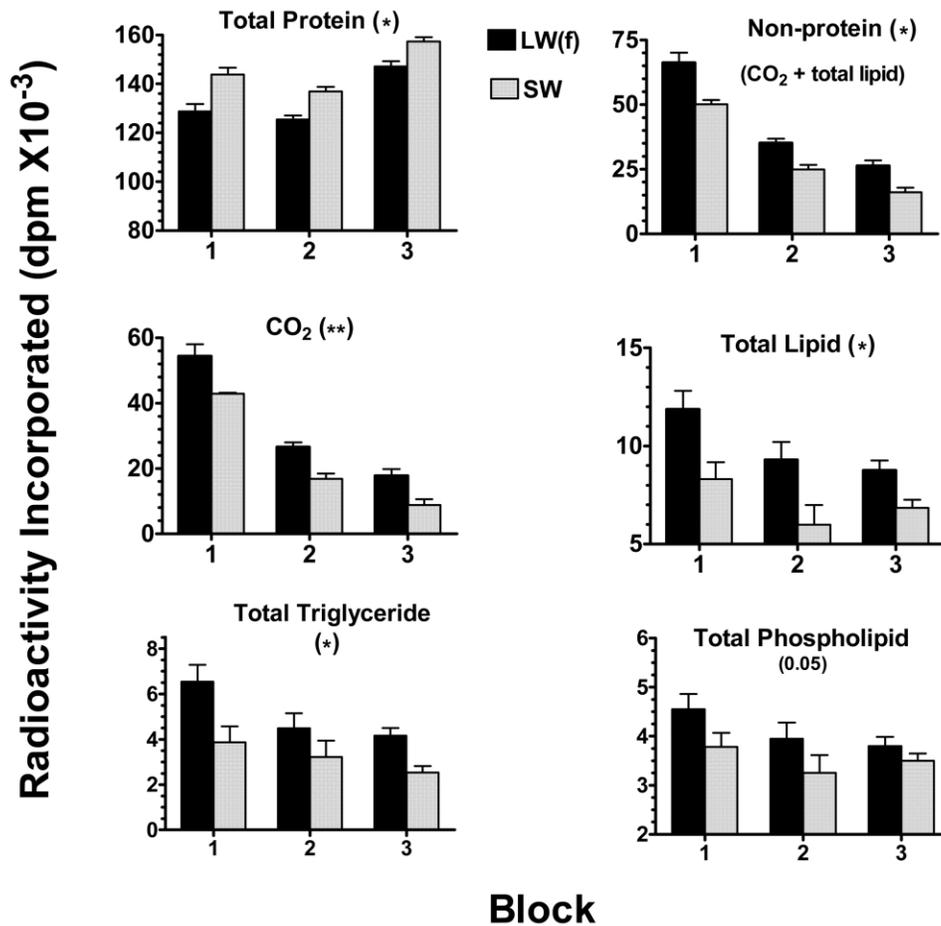


Figure 2: Whole-organism rates of metabolism of glycine into protein, CO_2 , total lipid, and major lipid components in three pairs (blocks) of LW(f)- and SW-selected lines of *Gryllus firmus*. Histograms represent mean \pm SEM radiolabel (disintegrations per minute [DPM]), derived from injected ^{14}C -glycine, that was incorporated into various metabolic end products during the 5-h incubation period. Means were based on assays of six to nine individuals per line, and incorporated DPM values were standardized to total incorporation by ANCOVA (see "Material and Methods"). These standardized DPM values are virtually identical to unstandardized values. Single asterisks indicate $P < .05$; double asterisks indicate $P < .01$. Significance is the result of paired *t*-tests of the three pairs of line means, indicating genetic differences in rates of incorporation between LW(f) and SW lines.

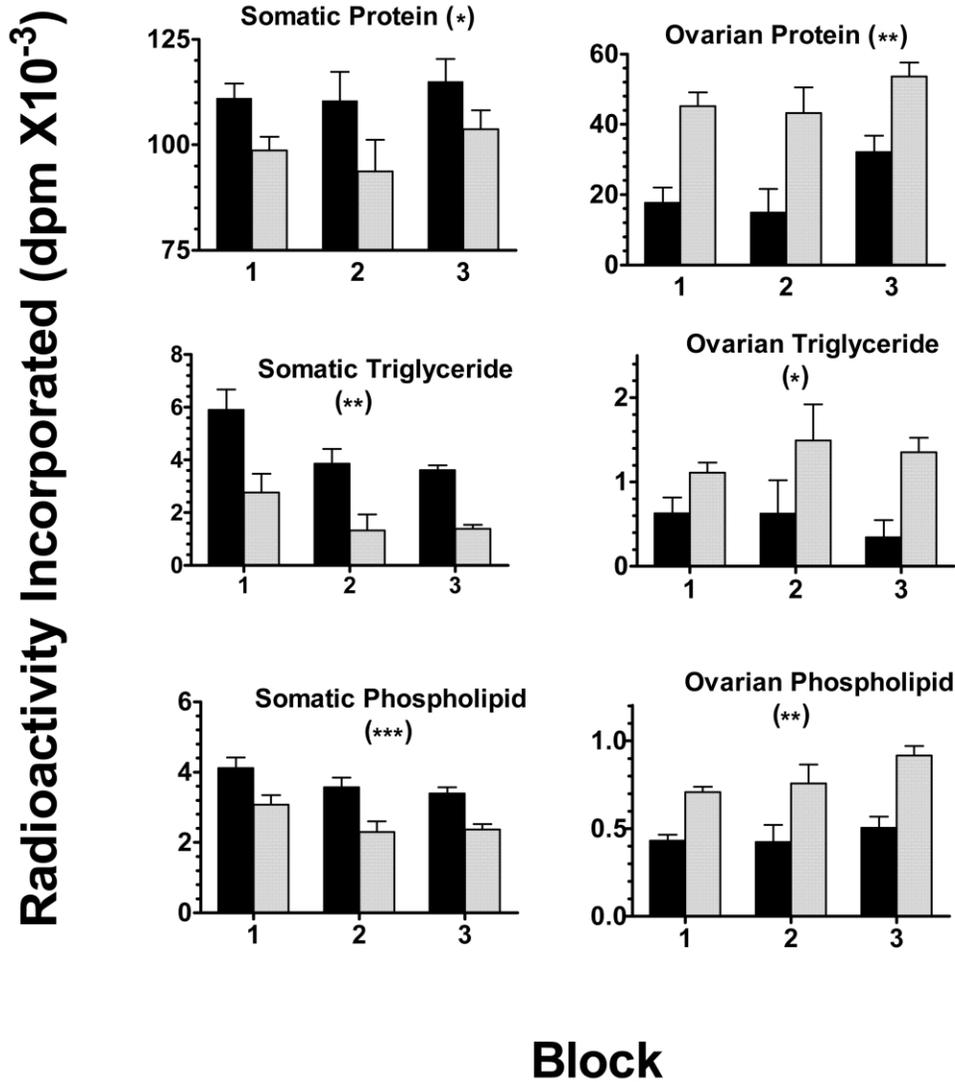


Figure 3: Incorporation of radiolabel from glycine into various end products of metabolism allocated to ovarian or somatic (whole body minus ovaries) body compartments during the 5-h incubation period. Triple asterisks indicate $P < .005$; other symbols and sample sizes are the same as those in figure 2.

offs. Because we simultaneously measured the incorporation of radiolabel from glycine into various chemical constituents and body compartments in all individuals, we could directly estimate and test for morph differences in metabolic and allocation trade-offs (i.e., morph differences in proportional metabolism or allocation of end products).

Significant genetic trade-offs of large magnitude were documented in four of six cases studied, while in another case, trade-off F, a nearly significant genetic trade-off was observed ($P = .06$; table 1). Most notably, LW(f) lines diverted 50% more radiolabeled carbon from ¹⁴C-glycine (24% as opposed to 16%) into nonprotein (CO₂ and lipid)

versus protein metabolites (branch point A in table 1 and fig. 1A) than did SW lines. LW(f) lines also diverted a proportionally greater amount of carbon from glycine into the biosynthesis of triglyceride (flight fuel) versus phospholipid (structural lipid especially important in developing eggs; branch point C in table 1 and fig. 1A). In addition, morphs traded off allocation of newly biosynthesized protein or lipid components to different body compartments in each of three trade-offs investigated (branch points D–F in table 1 and fig. 1B). LW(f) lines preferentially diverted newly biosynthesized protein and lipid components to the soma relative to SW lines.

Finally, trade-offs were not observed in all cases. LW(f) lines diverted a substantially greater amount of radiolabel from glycine through the Krebs cycle into both CO₂ and total lipid; however, the relative proportion of radiolabel diverted into these two metabolites did not differ between LW(f) and SW lines (trade-off B in fig. 1A and table 1 was not statistically significant).

Discussion

Although the differential diversion of nutrients through alternate pathways of metabolism is widely assumed to be a key component of life-history trade-offs, few studies have directly investigated the existence of this phenomenon in vivo, estimated the magnitude of trade-offs, or identified specific physiological or biochemical processes that are involved (Zera and Harshman 2001; O'Brien et al. 2002, 2005; Zhao and Zera 2002; Zera 2005). This is especially true for genetically based life-history trade-offs. One of the most important findings of this study was the ubiquity and large magnitude of genetically based trade-offs of internal resources in selected lines of *Gryllus firmus*. LW(f) and SW lines typically differed by 25% or more in the proportional allocation of radiolabeled carbon from glycine into alternate products of metabolism or to somatic versus reproductive organs. Importantly, these trade-offs occurred during the period of adulthood when the dramatic trade-off occurs between ovarian growth and triglyceride accumulation in alternate morphs (Zera and Larsen 2001). These results, combined with data from other recent studies on lipid metabolism in *G. firmus* (see below), strongly suggest that large-scale modifications of multiple, interacting pathways of intermediary metabolism are key components of the trade-off between early-age fecundity and flight capability in *G. firmus*.

As expected, SW females diverted a greater amount of ¹⁴C-glycine to biosynthesis of ovarian protein at the expense of somatic protein (fig. 3). Elevated biosynthesis of ovarian proteins such as vitellogenin is likely to be a major factor responsible for the greater ovarian growth in the SW morph, which occurs primarily by accumulation of protein into eggs. By contrast, LW(f) females diverted a greater amount of biosynthesized protein to somatic tissues, probably to the large flight muscles and the fat body, both of which are considerably larger in LW(f) than in SW morphs (Zera et al. 1997; Zera and Zhao 2003a). The higher rate of total protein biosynthesis from glycine in SW versus LW(f) morphs (fig. 2) suggests that total protein biosynthesis is not equivalent in the morphs and that the enhanced reproductive output of the SW morph requires more protein biosynthesis than does flight capability in the LW morph. To our knowledge, this is the first estimate of the relative costs of these two important organ-

ismal functions, in terms of protein biosynthesis, in any organism.

One of the most important findings of this study was the substantially greater genetically based oxidation of glycine by LW(f) than by SW females (fig. 2; table 1). This observation is noteworthy in light of the substantially greater (>100%) oxidation of fatty acid by SW than by LW(f) lines reported in an earlier study (Zera and Zhao 2003b). These studies collectively demonstrate remarkable morph-specific compensatory interactions between amino acid and lipid metabolism that result in the morph-specific sparing versus oxidation of specific nutrients (fig. 4). Increased oxidation of amino acids for energy in LW(f) females appears to preferentially spare fatty acids, allowing their enhanced conversion into triglyceride, the main flight fuel of this species. Conversely, increased oxidation of fatty acid by the SW morph appears to preferentially spare amino acids for enhanced conversion to ovarian protein. The enhanced oxidation of amino acids in the LW(f) morph not only spares lipid for flight, but it also probably provides the energy to fuel its elevated biosynthesis of lipid; similarly, the preferential oxidation of fatty acid in the SW

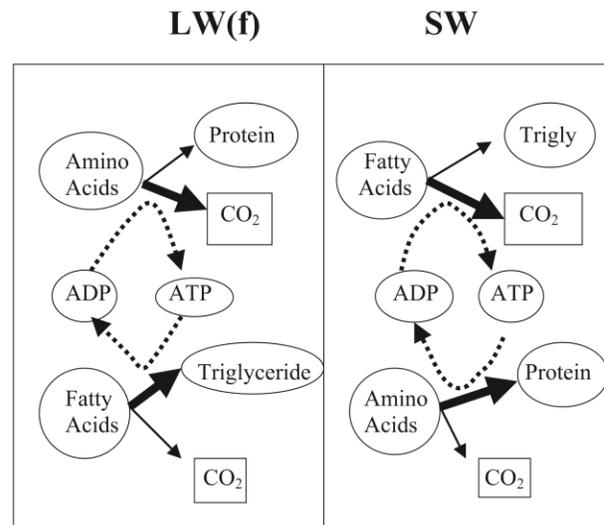


Figure 4: Morph-specific interaction between amino acid and lipid metabolism resulting in morph-specific sparing versus oxidation of amino acids versus lipid. Differences in the thickness of arrows at a branch point denote qualitative differences in proportional flux. Note that amino acids are preferentially oxidized and fatty acids are preferentially spared for conversion into triglyceride flight fuel in the LW(f) (flight-capable) morph. By contrast, fatty acids are preferentially oxidized and amino acids are preferentially spared for conversion into protein in the SW (flightless, reproductive) morph. Qualitative differences between morphs in amino acid and fatty acid oxidation are based on data from figure 2 and table 1 and from Zera and Zhao (2003b), respectively.

Table 2: Genetic differences in amino acid and lipid metabolism between flight-capable (LW(f)) and flightless (SW) morphs of *Gryllus firmus*

Aspect of metabolism	Activity in LW(f) morph relative to SW morph	References
Absolute biosynthesis of total lipid	Higher	Zhao and Zera 2001, 2002
Absolute biosynthesis of triglyceride	Higher	Zhao and Zera 2002
Absolute biosynthesis of phospholipid	Lower	Zhao and Zera 2002
Relative biosynthesis of triglyceride versus phospholipid	Higher	Zhao and Zera 2002
Oxidation of fatty acid	Lower	Zera and Zhao 2003b
Relative utilization of fatty acid for biosynthesis versus oxidation	Higher	Zera and Zhao 2003b
Specific activities of lipogenic enzymes	Higher	Zhao and Zera 2001; Zera and Zhao 2003a
Conversion of amino acids into lipid	Higher	This study
Oxidation of amino acid	Higher	This study
Activity of transaminases	Higher	Zhao and Zera 2001; Zera and Zhao 2003a
Biosynthesis of ovarian protein from amino acids	Lower	This study
Allocation of biosynthesized lipid to soma versus ovaries:		
Triglyceride	Higher	Zhao and Zera 2002; this study
Phospholipid	Lower	Zhao and Zera 2002; this study
Assimilation of lipid or protein from diet	Equivalent	Zera and Brink 2000

Note: Individuals were reared on the "standard" diet (see Zera and Larsen 2001 for diet composition). Flight-capable morph = long-winged individuals with large pink (functional) flight muscles; flightless morph = short-winged individuals with underdeveloped flight muscles.

morph probably provides the energy to drive the enhanced biosynthesis of protein required for the elevated reproductive output of the SW morph. To our knowledge, this is the first detailed information on interactions between different pathways of intermediary metabolism that underlie genetic adaptations to different life histories, either within or between species. It also is one of the most striking cases of adaptive divergence in intermediary metabolism among phenotypes within a species of which we are aware. These key metabolic differences could only have been identified by detailed *in vivo* studies of metabolism. Because the respiratory quotients of amino acid and lipid are approximately equivalent (Withers 1992), differences between the morphs in lipid versus amino acid oxidation would have gone undetected if metabolic differences between morphs had been compared using only whole-organ respirometry, a common approach in functional studies of life-history trade-offs (Zera and Harshman 2001).

LW(f) females also diverted a greater amount of amino acid through the Krebs cycle into lipid than did SW females. This greater diversion probably reflects the higher demand for triglyceride flight fuel (Zera et al. 1999) in the LW(f) morph (note that morphs did not fly during the study; the differential production of triglyceride reflects solely the preparation for flight). Indeed, LW(f) females preferentially converted radiolabeled carbon from glycine into triglyceride, as opposed to phospholipid (fig. 2); phospholipid is a structural lipid not used as a flight fuel. Previous studies have documented greater *de novo* bio-

synthesis of total lipid, greater proportional biosynthesis of triglyceride versus phospholipid from acetate or fatty acid, and reduced oxidation of fatty acid in LW(f) versus SW females (Zhao and Zera 2002; Zera and Zhao 2003a, 2003b; Zera 2005; table 2). Thus, the increased accumulation of triglyceride flight fuel in the LW(f) morph results from four important genetically based modifications of metabolism: increased *de novo* biosynthesis of fatty acids, greater proportional diversion of fatty acids into triglyceride versus phospholipid, reduced oxidation of lipid, and increased conversion of amino acids into lipid (fig. 3; table 1).

The higher rate of amino acid metabolism through the Krebs cycle by LW(f) lines observed in this *in vivo* study (figs. 1, 2) is supported by independent evidence obtained in a previous *in vitro* enzymological study (Zera and Zhao 2003a). In that study, LW(f) lines of *G. firmus* exhibited significantly higher *in vitro* activities of alanine and aspartate aminotransferases, enzymes that convert amino acids into Krebs cycle intermediates. The existence of elevated activities of multiple aminotransferases in LW(f) lines, which act on different classes of amino acids, also suggests that enhanced diversion of amino acids through the Krebs cycle probably occurs for amino acids in general, not only for glycine.

Variation in the allocation of nutrients to life-history traits can result from variation in the intake of external resources as well as from the differential biosynthesis of internal nutrients (Boggs 1997a; Zera and Harshman 2001;

see below). On the standard diet, used in this study, LW(f) and SW lines differ to only a small degree (<10%) in total food consumption or assimilation of total nutrients, protein, lipid, or carbohydrate from ingested food (Zera and Brink 2000; A. J. Zera, unpublished data). This situation is similar to that of flightless and flight-capable morphs in other *Gryllus* species, which do not differ significantly in nutrient consumption or assimilation (Mole and Zera 1993; Zera et al. 1998; Zera and Harshman 2001). Thus, the primary mechanism by which various chemical constituents such as protein and lipid are differentially allocated to reproductive versus somatic functions is by morph-specific metabolic conversions of nutrients following assimilation from the diet. Wing polymorphism in *G. firmus* is one of the only experimental models for which quantitative assessments have been made regarding the relative importance of variation in nutrient acquisition versus variation in internal nutrient biosynthesis to a life-history trade-off. Surprisingly, very limited data are available on nutrient acquisition in most experimental models used in physiological studies of life-history trade-offs (Zera and Harshman 2001), a notable exception being the studies by Boggs and coworkers (Boggs 1997a; Zera and Harshman 2001; O'Brien et al. 2002, 2005).

Recent studies of amino acid acquisition from the diet and allocation to egg production in lepidopterans (moths and butterflies) by Boggs and coworkers (Boggs 1992, 1997a; O'Brien et al. 2002, 2005) invite comparison with this study. Using stable isotopes and radioisotopes, these workers demonstrated that the extent to which amino acids required for egg production are derived from larval versus adult food can have a profound effect on total fecundity and the timing of egg production. An unexpected and significant finding of these studies is that egg production in species whose adults primarily feed on nectar (i.e., primarily sugar) may be more constrained by their inability to synthesize the complex carbon structures that make up essential amino acids than by the availability of nitrogen per se, the factor that has been most commonly thought to limit egg production in plant-feeding insects. Studies by Boggs and coworkers, which primarily focused on the role of variation in nutrient input in interspecific life-history trade-offs in an ecological context, complement our studies of genetically based variation in internal trade-offs of pathways of amino acid metabolism to a within-species life-history trade-off in an evolutionary context. These studies collectively illustrate the importance of detailed in vivo investigations of specific mechanisms of internal allocation and acquisition for understanding fundamental mechanistic aspects of the evolution and ecology of life histories.

Finally, this study and previous studies (e.g., Zera et al. 1997, 1998; Zera and Bottsford 2001; Zera and Cisper

2001; Zhao and Zera 2002; Zera and Zhao 2003b, 2004; Zera 2005; table 2) illustrate the exceptional power of wing polymorphism in *G. firmus* as an experimental model to investigate functional aspects of life-history evolution. Despite very low statistical power ($df = 2$ in statistical tests of genetic differences between LW(f) and SW lines; see "Material and Methods"), we were able to demonstrate genetic differences between LW(f) and SW lines of *G. firmus* in many aspects of lipid and amino acid metabolism (Zera and Larsen 2001; Zhao and Zera 2002; Zera and Zhao 2003a, 2003b; Zera 2005; figs. 2, 3; table 1). This is the consequence of the large-magnitude differences in intermediary metabolism, physiology, and life history that exist between morphs of *G. firmus*, which are as large or larger than those typically found between species.

Acknowledgments

This work was supported by grants from the National Science Foundation (IBN-9808249 and IBN-0516973) to A.J.Z.

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Associate Editor: Locke Rowe
 Editor: Jonathan B. Losos