University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Anthony Zera Publications

Papers in the Biological Sciences

October 1997

Flight-Muscle Polymorphism in the Cricket *Gryllus firmus*: Muscle Characteristics and Their Influence on the Evolution of Flightlessness

Anthony J. Zera

University of Nebraska - Lincoln, azera1@unl.edu

Jeffry Sall

University of Nebraska - Lincoln

Kimberly Grudzinski

University of Nebraska - Lincoln

Follow this and additional works at: <https://digitalcommons.unl.edu/bioscizera>

Part of the [Microbiology Commons](#)

Zera, Anthony J.; Sall, Jeffry; and Grudzinski, Kimberly, "Flight-Muscle Polymorphism in the Cricket *Gryllus firmus*: Muscle Characteristics and Their Influence on the Evolution of Flightlessness" (1997). *Anthony Zera Publications*. 5.

<https://digitalcommons.unl.edu/bioscizera/5>

This Article is brought to you for free and open access by the Papers in the Biological Sciences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Anthony Zera Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Flight-Muscle Polymorphism in the Cricket *Gryllus firmus*: Muscle Characteristics and Their Influence on the Evolution of Flightlessness

Anthony J. Zera*

Jeffrey Sall

Kimberly Grudzinski

School of Biological Sciences, University of Nebraska,
Lincoln, Nebraska 68588

Accepted by C.P.M. 1/9/97

ABSTRACT

Flight muscles of the cricket *Gryllus firmus* are polymorphic, existing as pink or white phenotypes. White muscles are smaller in size, have reduced number and size of muscle fibers, and have reduced in vitro enzyme activities and respiration rates relative to pink muscles of newly molted, fully winged adults. *G. firmus* is also polymorphic for wing length. All newly molted long-winged adults exhibited the pink-muscle phenotype, while most newly molted short-winged adults exhibited the white-muscle phenotype, which resulted from arrested muscle growth. As long-winged adults aged, fully grown pink muscle was transformed into white muscle via histolysis. The substantially higher respiration rate of pink muscle likely contributes to the elevated whole-organism respiration rate of long-winged females, which has been documented previously and which is thought to divert nutrients from egg production. Histolyzed white flight muscle from long-winged crickets also exhibited significantly elevated respiration rate and enzyme activities compared with underdeveloped white muscle from short-winged adults, although these differences were not as great as those between pink and white muscles. Fecundity was much more elevated in females with white versus pink flight muscles than it was in females with short versus long wings. The fitness gain resulting from flightlessness has typically been estimated in previous studies by comparing enhanced egg production of short-winged and long-winged females, without considering the influence of flight-muscle variation. Our results suggest that the magnitude of this fitness gain has been substantially underestimated.

Introduction

Wing polymorphism in insects has been extensively studied from physiological, genetic, and ecological perspectives to iden-

tify the factors that affect dispersal in natural populations (Harrison 1980; Dingle 1985; Pener 1985; Roff 1986; Zera and Mole 1994; Zera and Denno 1997). An important finding of these studies is that dispersal capability has physiological and fitness costs. Fully winged females typically begin egg development later and have reduced fecundity relative to flightless (short-winged or wingless) females (Pener 1985; Roff 1986; Zera and Denno 1997). This observation, together with more recent physiological comparisons of wing morphs, indicates that the attainment and maintenance of flight capability involves a significant energetic cost, which reduces egg production (Tanaka 1993; Zera and Mole 1994; Zera and Denno 1997).

Although wing polymorphism derives its name from variation in wing length, wing morphs also differ in flight-muscle characteristics. Flight muscles are reduced in the flightless morph (Smith 1964; Pener 1985; Roff 1986). Muscle reduction is thought to be more important than wing reduction in elevating the fecundity of the flightless morph (Zera and Denno 1997); however, there is a paucity of anatomical, physiological, and biochemical data on flight muscles in wing-polymorphic insects (reviewed in Smith [1964] and Pener [1985]). This paucity of information limits our understanding of the physiological mechanisms underlying the trade-off between flight capability and reproduction and has also likely resulted in an underestimate of the fitness cost of flight capability.

The fitness cost of flight capability has typically been assessed by comparing the decrease in fecundity of long-winged females that have not flown with that of short-winged females of wing-polymorphic insects (Harrison 1980; Roff 1986; Zera and Denno 1997). However, it has long been recognized that long-winged individuals of the same age often exhibit dramatic variation in flight-muscle status. Some long-winged females exhibit fully developed flight muscles when reproduction commences, while others have histolyzed flight muscles at this time and thus are functionally flightless (Young 1965; Pener 1985). Flight-muscle polymorphism within the long-winged subpopulation would result in an underestimate of the costs of flight capability if flight-muscle histolysis elevates fecundity and if long-winged individuals with and without histolyzed flight muscles are not distinguished. The extent to which this occurs is unknown.

The present study is the first in a series of investigations that focuses on flight-muscle characteristics of long-winged and short-winged morphs of the wing-dimorphic cricket, *Gryllus firmus*. This species and its congener *Gryllus rubens* have been used in physiological studies on the trade-off between flight capability and fecundity (Mole and Zera 1993; Zera and Mole

*To whom correspondence should be addressed; E-mail: azera@unlinfo.unl.edu.

1994; Zera et al. 1994). Prior to the present study, only rudimentary information was available on any aspect of flight-muscle variation in these species. The present study had three goals. The first was to obtain baseline information on the morphology, development, and physiology of the various flight-muscle phenotypes in the long-winged and short-winged morphs. The second was to determine the degree to which reduced flight-muscle phenotypes that are produced by different processes (arrested growth in juveniles vs. histolysis in adults) resemble each other in morphology, physiology, and impact on egg production. The degree to which this occurs is expected to have an important influence on the evolution of flightlessness (see Discussion). The third goal was to assess the degree to which enhanced fecundity is associated with reduced flight muscles rather than reduced wing length and the consequences of this on estimates of the cost of dispersal capability.

Material and Methods

Insect Rearing

Gryllus firmus, the sand cricket, occurs in the southeastern United States as a long-winged morph, some of which are capable of flight, or as a short-winged form that is obligately flightless (Veazy et al. 1976). The *G. firmus* used were from the same laboratory colonies that were used in previous physiological studies (Zera and Mole 1994; Zera et al. 1994). Standard rearing conditions (28°C, 16L : 8D photoperiod, 40–60 adults per 38-L aquarium, no egg-laying substrate) are detailed in those reports.

Developmental Profiles of Flight Muscles and Reproductive Organs

Size, color, and wet weight of the flight muscles (dorsolongitudinal and dorsoventral muscles) were determined for long-winged and short-winged adult females (ages 1, 3, 5, 7, and 9 d) and males (ages 1, 5, 9, and 11–13 d after adult eclosion). Whole-cricket weights were also obtained at the time of flight-muscle dissections. Dorsolongitudinal and dorsoventral muscles, along with a small amount of attached cuticle, were dissected free from fat bodies and other attached tissues and weighed. The small amount of attached cuticle was ignored, since it is difficult to remove and because it contributes less than 6% to the total wet weight (Mole and Zera 1993).

Testes or ovaries were removed from the same crickets from which flight muscles were obtained. Reproductive organs were weighed, and in the case of ovaries, the number of postvitellogenic eggs (see Zera et al. 1993) was counted in one ovary and multiplied by two to obtain the total number of eggs per female. In addition to obtaining developmental profiles of flight muscles and female reproductive characteristics of crickets reared under standard conditions (defined above), we also obtained profiles on a separate group of females referred to as group 2

crickets. These crickets were reared singly with oviposition material in which they oviposited their eggs.

Histology

Dorsolongitudinal flight muscles were dissected out of long-winged or short-winged crickets of various ages, fixed in Bouin's fluid for 12 h, and dehydrated and infiltrated with paraffin. Cross sections (7 μ m) through the thickest portion of the muscles were stained with Masson's Triple Stain (Weesner 1960) and were examined under a light microscope. Muscle fibers were oblong and mean fiber cross-sectional area per muscle was calculated as follows: Maximum and minimum fiber diameters were measured for each of six randomly chosen fibers. The average cross-sectional area was computed as the product of the average maximum and minimum diameters. The total number of fibers per muscle was calculated by dividing the cross-sectional area of the entire muscle by the average fiber area.

Isocitrate Dehydrogenase and Citrate Synthase Activities

Dorsolongitudinal flight muscles were dissected out of long-winged or short-winged crickets of various ages and assayed for NADP⁺-dependent isocitrate dehydrogenase (IDH, EC 1.1.1.42) or citrate synthase (CS, EC 4.1.3.7) activity. These enzymes were chosen to represent typical cytoplasmic and mitochondrial enzymes of intermediary metabolism. CS activity is also an indicator of mitochondrial volume and aerobic capacity in insect flight muscle (Ready and Najam 1985). Dissected muscle was blotted dry, weighed and homogenized in 20 vol of 3-(N-morpholino)propanesulfonic acid buffer (ionic strength = 0.1), pH 7.1. The homogenate was centrifuged at 13,000 g for 10 min and the supernatant was assayed for IDH activity as described in Bergmeyer (1974), or the homogenate was diluted and assayed directly for CS activity as described in Craig (1973). Enzyme assays were conducted at 30°C under conditions such that the change in absorbance was linearly related to assay time (the linear portion of the progress curve). Preliminary experiments also documented that activity was proportional to enzyme (homogenate) concentration.

Basal Respiration Rates of Thoracic Muscles

The entire thoracic musculature (dorsolongitudinal plus dorsoventral muscles) plus a small amount of attached cuticle was quickly dissected out of day 1–8 long-winged or short-winged females and weighed. Care was taken to remove all fat bodies and to keep the muscles in a moist environment until respiration rates were measured (within 10 min of the start of each dissection). Single thoraces from long-winged females or pairs of thoraces from short-winged females were placed on a moist piece of cotton inside a glass chamber of a Micro-Oxymax respirometer (Columbus Instruments). O₂ uptake and CO₂

production were measured every 6 min over a period of 18 min at 28°C. Respiratory quotients were calculated in the standard fashion as the ratio of CO₂ production over O₂ uptake. Background experiments documented that O₂ uptake and CO₂ production were linear during the measurement period, that the moist cotton did not contribute to the respiration rate, and that respiration rates were less variable among samples of the same tissue type and were linear for a longer period of time when thoraces were placed on moist cotton than when they were submerged in various tissue-culture media.

Statistics

Frequency data were analyzed by χ^2 contingency tests. Means of physiological and anatomical traits were analyzed by ANOVA (Sokal and Rohlf 1981). In cases where distributions did not conform to the requirements of ANOVA (e.g., unequal variances), the nonparametric Kruskal-Wallis (K-W) test was used (Sokal and Rohlf 1981). Comparisons between respiration rates and enzyme activities of pink and white muscle were one-tailed, on the basis of the a priori expectation that these features would be reduced in white muscle. This expectation is based on several studies of wing-monomorphic insects that have documented reduced protein or cytochrome content in histolyzed muscles compared with fully developed muscles (see Discussion). We had no a priori expectations concerning the direction of differences between the various flight-muscle morphs for respiratory quotients. Consequently, tests of this characteristic were two-tailed. For the same reason, tests comparing features of the two white-muscle types (histolyzed and underdeveloped) also were two-tailed. In cases in which the same data were used for multiple comparisons, the probability values given are adjusted for experiment-wide error by the Bonferroni method (probabilities were multiplied by the number of comparisons performed; Dunn 1959).

Results

Variation in Flight-Muscle Morphology with Age and Wing Morph

The thoracic musculature of *Gryllus firmus* (dorsolongitudinal and dorsoventral muscles) exhibited discontinuous variation in every anatomical and physiological trait studied. Thoracic muscles were either large (8%–14% of total body mass) and bright pink or were small and white (4%–8% of total body mass) (Fig. 1). Pink muscle exhibited both larger fiber size and a greater number of muscle fibers than white muscle (Table 1). Neither the pink nor the white phenotype exhibited any temporal change in muscle mass as a percentage of total body weight. Linear regressions of arcsine-transformed percentage muscle mass on age were not significant for either pink or white muscle from either males or females ($P > 0.1$ for all regressions).

Figure 1. Variation in flight-muscle mass among flight-muscle morphs and wing morphs as a function of adult age. A, females; B, males. PK and WT = pink and white muscles, respectively. LW and SW = long wings and short wings, respectively. Values are means, and bars represent standard errors. Sample sizes are the same as those in Table 2 and did not include group 2 females. Age refers to days after adult eclosion.

In a number of features, white or pink muscles of one wing morph differed from the same muscle type found in the alternate wing morph. In each of seven comparisons, white or pink muscles in short-winged adults were smaller than their counterparts in long-winged adults of the same age and sex (Fig. 1). White muscles in the short-winged morph of *G. firmus* also lacked the fibrous appearance of white muscles in the long-winged morph. The tracheolar network of white muscles in short-winged crickets was more visible under a dissecting microscope than the network in white muscle in long-winged crickets of the same age, indicating a greater degree of tissue reduction. Pink muscles in the short-winged morph were also less pink than their long-winged counterparts. Thus, pink muscles never develop as fully in the short-winged morph as they do in the long-winged morph, while full development of pink flight muscles in the long-winged morph seems to prevent these muscles from ever attaining the degree of reduction found in white muscles of the short-winged morph, which never fully develop.

The frequency of pink and white phenotypes varied with age, sex, wing morph, and reproductive status. All newly molted long-winged individuals had pink muscles, while the

Table 1: Cross-sectional area and number of fibers in pink muscles from long-winged and in white muscles from short-winged *Gryllus firmus*

Age ^a	Fiber Area (μm ²)		Fiber Number	
	Pink	White	Pink	White
1–2	383 ± 16 (n = 3)	74 ± 24 (n = 3)	2,518 ± 458 (n = 3)	1,398 ± 316 (n = 3)
10–11	438 ± 59 (n = 2)	58 ± 3 (n = 2)	2,728 ± 91 (n = 2)	1,645 ± 3 (n = 2)
Pooled	405 ± 23 ^b (n = 5)	68 ± 13 ^b (n = 5)	2,602 ± 288 ^c (n = 5)	1,497 ± 205 ^c (n = 5)

Note. Values are means ± SE, with number of individual crickets studied in parentheses.
^aDays after adult ecdolosis.
^bMeans are significantly different from each other (*t*-test, *P* < 0.001).
^cMeans are significantly different from each other (*t*-test, *P* < 0.005).

majority (70%) of newly molted short-winged individuals had white muscles (Table 2; Fig. 1). By day 5 and thereafter, all short-winged crickets exhibited only white muscles. Long-winged *G. firmus* began to exhibit the white-muscle phenotype on day 5, and this phenotype became increasingly common with age. In both males and females, the frequency of the pink-muscle phenotype was higher in the long-winged morph than in the short-winged morph in each age class (Table 2). The

exception was the oldest crickets, for which the frequency of the white-muscle phenotype did not differ between morphs of either sex. A similar pattern of flight-muscle development was seen in group 2 long-winged and short-winged females (Table 2), which were reared under conditions different from those of the standard crickets (see Material and Methods). Finally, developmental changes in the color of the flight muscles were similar in males and females of the same wing morph except for the earlier appearance of the white-muscle phenotype in long-winged females.

Table 2: Number of long-winged and short-winged adult *Gryllus firmus* with pink- and white-muscle phenotypes as a function of age

Age (d)	Wing Morph				χ^2 ^b
	Long-Winged		Short-Winged		
	Pink ^a	White	Pink ^a	White	
Males:					
1	7	0	4	6	4.71*
5	7	2	0	7	7.69**
9	6	0	0	6	9.75***
11–13	2	9	0	14	.86, N.S.
Females: ^c					
1–3	13 (13)	0 (0)	5 (3)	9 (11)	34.34***
5–7	9 (11)	6 (4)	0 (0)	18 (13)	32.22***
9	2 (6)	6 (4)	0 (0)	8 (7)	7.71**
12	(3)	(6)	(0)	(14)	2.86, N.S.

^aMuscle color was very light pink in two short-winged day 1 males, one long-winged day 9 male, and six short-winged day 1–3 females. These muscles were classified as pink.
^bResults of χ² tests: * = *P* < 0.05; ** = *P* < 0.01; *** = *P* < 0.005; N.S. = not significant.
^cNumbers in parentheses refer to flight-muscle morphs observed in a separate group (group 2) of female crickets; see Material and Methods for rearing conditions. χ² tests of female muscle phenotypes were performed on pooled data for the two sets of females. See text for description of muscle phenotypes.

IDH and CS Activities

Activity of cytoplasmic IDH was greater in pink dorsolongitudinal flight muscle from long-winged crickets than in white muscle from short-winged crickets of comparable ages for each of the three age classes studied (Table 3). This was the case for whole-muscle activity (typically six- to 10-fold higher) and for specific activity (activity/unit muscle mass; typically three- to fourfold higher; *P* < 0.01 in all ANOVAs). IDH activity was significantly lower in white than in pink dorsolongitudinal muscle from long-winged individuals on days 7–11 for whole-muscle activity (ANOVA, *P* < 0.01) but not for specific activity (ANOVA, *P* > 0.1). This was the only age-group in which a comparison could be made between different flight-muscle types within long-winged crickets. White muscle in long-winged crickets always exhibited higher IDH activity than white muscle in short-winged animals of the same age (whole-muscle or specific activity; *P* < 0.02 for all ANOVAs).
Activity of the mitochondrial enzyme CS exhibited even more dramatic variation among muscle types (Table 4). Whole-muscle activity of pink muscle from long-winged crickets was 30-fold higher than that of white muscle from long-winged animals (K-W test, *P* < 0.05) and 70-fold higher than that of white muscle from short-winged crickets (K-W test, *P* < 0.05). The specific activity of CS for pink muscle from

Table 3: NADP⁺-dependent IDH activities in white and pink dorsolongitudinal flight muscles from long-winged and short-winged *Gryllus firmus* of various ages

Wing Morph (Age)	Muscle Phenotype	Whole-Muscle Activity (nmol min ⁻¹)	Wet Weight of Muscle (mg)	Activity (mg ⁻¹ Muscle)
Long-winged (day 0–6)	Pink	48.8 ± 4.4	9.0 ± .9	5.4 ± .9
Short-winged (day 0–6)	White	7.6 ± .5	5.2 ± .2	1.5 ± .1
Long-winged (day 7–11)	Pink (<i>n</i> = 3)	54.5 ± 2.3	10.6 ± .5	5.1 ± .4
	White (<i>n</i> = 3)	34.2 ± .8	7.7 ± .4	4.5 ± .3
Short-winged (day 7–11)	White	5.8 ± .5	4.2 ± .3	1.4 ± .0
Long-winged (> day 12)	White	15.3 ± 1.0	7.2 ± .4	2.1 ± .2
Short-winged (> day 12)	White	4.4 ± .2	3.1 ± .2	1.4 ± .0

Note. Except where noted, all values are means (±SE) of five animals.

long-winged individuals was sixfold higher than that for white muscle from either long-winged or short-winged crickets ($P < 0.05$ for each K-W test). Neither specific activity nor whole-muscle activity differed between white muscle from long-winged and short-winged animals ($P > 0.1$ for each K-W test).

Basal Respiration Rates of Thoracic Muscles

Whole-thorax respiration rate was reduced by 39% (O₂ uptake) or by 55% (CO₂ production) for thoraces from long-winged crickets containing white rather than pink muscle. Respiration rate was further reduced by 47% (O₂ uptake) or 35% (CO₂ production) for thoraces containing white muscle from short-winged rather than from long-winged crickets (Table 5). In pairwise comparisons, whole-thorax O₂ or CO₂ respiration rates differed significantly between each of the three muscle types (K-W tests, $P < 0.025$ in each case). When respiration rates were standardized to rate per milligram of thoracic muscle, O₂-specific respiration rate differed between pink muscle from long-winged and white muscle from short-winged crickets (K-W test, $P < 0.05$). However, respiration rates did not differ between pink and white muscle from long-winged crickets

or white muscle from long-winged rather than short-winged crickets ($P > 0.1$ for each K-W test). CO₂-specific respiration rates were significantly reduced (ca. 30%–45%) in white muscle from either long-winged or short-winged crickets relative to pink muscle from long-winged animals ($P < 0.005$ for each K-W test). However, respiration rates did not differ between white muscle from long-winged and short-winged animals (K-W test, $P > 0.1$). Respiratory quotients were significantly lower in white muscle (from either long-winged or short-winged individuals) relative to pink muscle from long-winged individuals (K-W test, $P < 0.025$) but did not differ between white muscle from either wing morph (K-W test, $P > 0.1$; Table 5).

Reproductive Features of Wing and Flight-Muscle Morphs

Ovarian mass (% body mass) and total number of eggs (eggs oviposited plus postvitellogenic eggs in the ovaries) as a function of age are presented for the three wing and flight-muscle morphs of *G. firmus* (Fig. 2A, B). Two distinct reproductive profiles were evident that differed in the onset of ovarian growth and egg production. These two profiles were strongly correlated with flight-muscle phenotype but not with wing-length phenotype. All individuals that exhibited earlier reproduction had white muscles, but these early reproducers were a mixture of long-winged and short-winged females. No significant differences were observed in ovarian weight or egg number on any day of adulthood between crickets that differed in wing length but that had white muscles (K-W tests, $P > 0.1$). The one exception was day 10 females, in which long-winged individuals with white muscles produced significantly more eggs than short-winged females with white muscles.

In contrast to the dramatic variation in ovarian mass and egg number between flight-muscle morphs of *G. firmus*, we observed no analogous differences in testes weight between males of any age class with different flight muscles (Fig. 2C; $P > 0.1$, all K-W tests). Furthermore, testes weight decreased in

Table 4: CS activities in pink and white flight muscles from long- and short-winged *Gryllus firmus*

Morph	Specific Activity (nmol min ⁻¹ mg ⁻¹ muscle)	Whole-Muscle Activity (nmol min ⁻¹)
Pink/long-winged	102.2 ± 19 (<i>n</i> = 4)	1530 ± 437 (<i>n</i> = 4)
White/long-winged	16.5 ± 7.4 (<i>n</i> = 3)	50.1 ± 5.9 (<i>n</i> = 3)
White/short-winged	15.6 ± 4.9 (<i>n</i> = 3)	22.0 ± 9.1 (<i>n</i> = 3)

Note. *n* = number of animals assayed.

Table 5: Respiration rates of pink and white thoracic muscles from long-winged and short-winged *Gryllus firmus*

	Morph		
	Pink/Long-Winged	White/Long-Winged	White/Short-Winged
Sample size	8	6	6
Muscle mass (mg)	73 ± 3.4	54 ± 3.1	29 ± 2.3
Respiration rate (at 28°C):			
O ₂ uptake (nL min ⁻¹ mg ⁻¹ muscle)	33.8 ± 2.8	27.7 ± 2.7	28.7 ± 3.7
O ₂ uptake (whole thorax, nL min ⁻¹)	2,563 ± 326	1,564 ± 198	825 ± 25
CO ₂ release (nL min ⁻¹ mg ⁻¹ muscle)	28.6 ± 3.3	15.3 ± 0.7	19.3 ± 2.7
CO ₂ release (whole thorax, nL min ⁻¹)	1,909 ± 239	852 ± 35	552 ± 36
Respiratory quotient ^a	.90 ± .07	.57 ± .06	.67 ± .02

Note. Values are means ± SE.

^aCO₂ production/O₂ uptake.

size during the first 11 d of adulthood, in contrast to the dramatic increase in ovarian weight during this time period. The decrease was statistically significant in long-winged males with pink muscles (linear regression, $P < 0.05$) and was nearly significant in short-winged males with white muscle ($P < 0.08$).

The number of eggs produced by wing or flight-muscle morphs is given in Table 6. Comparisons between wing morphs (i.e., egg counts pooled for long-winged females with white and pink muscles) documented significantly greater egg production by the flightless, short-winged morph on days 5 and 7 but not on day 9. Comparisons between flight-muscle morphs (eggs from long-winged and short-winged females with white muscles pooled) resulted in a similar pattern of increased fecundity by the flightless, white-muscle morph. However, the number of eggs produced by the flightless morph was approximately 50% greater when flightlessness was defined by flight-muscle phenotype (white vs. pink) rather than wing-length phenotype (long wing vs. short wing).

Discussion

Morphological Aspects of Flight-Muscle Polymorphism in Gryllus firmus: Similarities with Other Insects

Results of the present study confirm that basic morphological and developmental features of flight-muscle variation in *Gryllus firmus* are similar to features reported in other wing-polymorphic insects (reviewed by Smith [1964]; Pener [1985]; Roff [1986]; Zera and Denno [1997]). All long-winged *G. firmus* molt into adults with large, pink flight muscles (Fig. 1; Table 2). These pink muscles begin to histolyze at the onset of ovarian growth in females, thus transforming the pink phenotype into the white phenotype. By contrast, most short-winged individuals molt into adults with underdeveloped flight muscles that are colorless and that are smaller and have smaller and fewer muscle fibers than pink muscle (Tables 1 and 2; Fig. 1). White

muscles in short-winged adults undergo no further reduction in mass with age (Fig. 1).

Although not investigated in *G. firmus*, the absence of color in white muscle is almost certainly due to reduced cytochrome content, which has been documented previously for histolyzed flight muscle in *Gryllus bimaculatus* (Shiga et al. 1991; Gomi et al. 1995). This reduced cytochrome content likely contributes to the lower respiration rate of white muscle relative to pink muscle in *G. firmus* (Table 5). The number of fibers in pink flight muscle from long-winged *G. firmus* (Table 1) is similar to the number reported previously for fully developed flight muscle from the closely related cricket *Acheta domestica* (Chudakova and Bocharova-Messner 1968). The 40% reduction in the white thoracic muscles of *G. firmus* is similar to the degree of muscle reduction reported for the analogous morph of *Gryllus rubens*, the only other insect species for which comparable quantitative data are available on reduction of the entire thoracic musculature in the flightless morph (Zera and Mole 1994). An even greater reduction in mass or volume of various individual histolyzed flight muscles has been reported for several other insects (e.g., up to 90% reduction; Borden and Slater 1969; Shiga et al. 1991; Tanaka 1994). Since we observed only a few intermediates between the pink- and white-muscle phenotypes in long-winged *G. firmus*, the transition between fully developed and histolyzed flight muscles must occur fairly rapidly. The actual mechanism of flight-muscle histolysis in insects is poorly understood except that the gonadotrophin juvenile hormone is likely involved (e.g., Pener 1985; Tanaka 1986, 1994; Kobayashi and Ishikawa 1994a, 1994b; Zera and Denno 1997). Finally, we have no information concerning the degree to which flight-muscle variation in our stocks of *G. firmus* results from genetic rather than environmental variation. However, Fairbairn and Roff (1990) documented a genetic basis for flight-muscle histolysis in other stocks of *G. firmus*. We therefore expect that flight-muscle variation also has a genetic basis in our stocks, and we are currently investigating this issue via artificial selection.

Figure 2. Variation in ovarian mass (A), number of postvitellogenic eggs (B), and testes mass (C) among flight muscle morphs and wing morphs as a function of adult age. Symbols are the same as those in Figure 1. Values are means and bars represent standard errors. Sample sizes for data in panels A and C are the same as those in Table 2 and did not include group 2 females. Means in panel B are based on five to 13 animals, except for white, long-winged females on day 7, where $n = 4$. Ovarian weights were subtracted from whole-body weights prior to calculation, since ovarian weights differed substantially with age, wing, and flight-muscle morph. Similar profiles were obtained when ovarian weights were not subtracted. For clarity, standard errors have been omitted from panel C.

Biochemical and Physiological Characteristics of Flight-Muscle Phenotypes

The present study extends previous anatomical investigations of flight-muscle polymorphism by documenting substantially reduced maximum enzyme activities and basal respiration rates for either histolyzed or underdeveloped white muscle compared with pink muscle of long-winged *G. firmus* (Tables 3–5). To our knowledge, there are no other comparisons of bio-

chemical properties of fully developed and reduced flight muscle in wing or flight-muscle polymorphic insects. The one exception is the cockroach *Periplaneta americana*, which exhibits sexual dimorphism for flight-muscle development. Similar to the results obtained for *G. firmus*, the results obtained for white underdeveloped flight muscle of female *P. americana* show reduced activities of mitochondrial enzymes and reduced oxygen uptake relative to pink flight muscle of the same age from males (Stokes et al. [1994] and references therein). A few studies, such as that by Chudakova and Gutmann (1978) on *A. domestica*, have documented reduced activities of mitochondrial enzymes in histolyzed white flight muscle of older reproducing females relative to pink flight muscle of prereproductive females. Results of these studies clearly document that white muscle is not simply a smaller version of pink muscle with similar metabolic features. Indeed, white and pink muscles in *G. firmus* appear to use different energy sources for basal respiration (Table 5; see below). In *G. firmus*, whole-muscle enzyme activities and whole-thorax respiration rates differ among the three muscle phenotypes to a much greater degree than do specific activities and specific respiration rates (Tables 3–5). Thus, the overall reduction in whole-thorax basal metabolism of white flight muscle is attained by both alteration of its metabolic characteristics and reduction of its mass. Finally, these data not only demonstrate that pink and white muscle in *G. firmus* differ physiologically, they also show that the two white-muscle types (histolyzed and underdeveloped) differ in nearly every whole-thorax physiological trait measured.

Information on flight-muscle enzymology and respiration obtained in the present study provides additional insight into the physiological processes underlying the energetic cost of flight capability and the trade-off between flight capability and reproduction in *Gryllus* species (Zera and Mole 1994; Zera and Denno 1997). In previous nutritional studies, we indirectly estimated that whole-cricket respiration (CO_2 production) is higher in long-winged than in short-winged females of *G. firmus* and *G. rubens* (Mole and Zera 1993; Zera and Mole 1994). This higher respiratory metabolism is thought to be a major contributor to the reduced conversion of assimilated nutrients into biomass in long-winged individuals. This, in turn, either results in reduced fecundity in the long-winged morph relative to the short-winged morph (*G. rubens*) or requires that the long-winged morph consume more nutrients to match the fecundity of short-winged females (*G. firmus*) (Zera and Mole 1994; Zera and Denno 1997). In our previous studies, we had no information concerning the source of the elevated, whole-cricket respiration in long-winged *G. firmus* or *G. rubens*. We speculated that this was most likely due to either or both of the following: a basal respiration rate of the large pink flight muscles found in the majority of long-winged adults that is higher than that found in the small white muscles of nearly all short-winged individuals or elevated lipid biosynthesis in long-winged adults (long-winged adults have 38% higher triglyceride content than short-winged adults; Zera and Mole

Table 6: Increased gain in egg production due to flightlessness, where flightlessness is defined by flight-muscle versus wing-length phenotype

	Wing Morph			Flight-Muscle Morph			Increased Gain in Egg Production ^c (%)
Age (d)	Long-Winged	Short-Winged	% Change ^a	Pink	White	% Change ^b	
Standard females:							
5	22	76	245	16	76	375	53
7	60	172	186	46	178	287	54
9	180	156	−13	160	166	4	130
Group 2 females:							
5	18	88	388	12	78	555	43
7	42	119	183	30	110	266	45
9	157	102	−35	95	132	40	132

Note. Values are median number of eggs in both ovaries (standard females) or median number of eggs in both ovaries plus oviposited eggs (group 2 crickets); see Material and Methods for further details. Sample sizes are given in Table 2, and data for standard females are presented in Fig. 2.

^aPercent change in egg production in short-winged vs. long-winged females (quantity *B* defined in footnote c).

^bPercent increase in egg production in white-muscle vs. pink-muscle females (quantity *A* defined in footnote c).

^cIncreased gain in egg production was calculated as $100(A - B)/B$, where $A = (WT - PK)/PK$, $B = (SW - LW)/LW$, and WT, PK, SW, and LW refer to median number of eggs in white-muscle, pink-muscle, long-winged, and short-winged phenotypes, respectively.

1994; Zera et al. 1994). Results of the present study directly document a threefold higher in vitro basal respiration rate of the thoracic musculature of long-winged individuals, which comprises a significant proportion (9%–14%) of total body mass (Fig. 1). These data add further credence to the notion that the reduced respiration rate of short-winged females results, at least in part, from reduced basal metabolism of their flight muscles. An analogous situation has been reported in birds. Flightless species with reduced pectoral muscles have reduced whole-organism metabolic rate compared with their flight-capable counterparts from the same taxon (McNab 1994). El-Ibrashy (1965) also has argued that histolysis of flight muscles in the Colorado potato beetle prior to overwintering is an adaptation to reduce metabolic rate.

The effect of elevated respiration of pink thoracic muscles on whole-organism respiration of long-winged *G. firmus* conceivably could be offset by reduced respiration of other tissues of this morph. However, available data indicate that the opposite situation likely occurs. Ovaries, which exhibit a 10-fold lower respiration rate than flight muscle (A. J. Zera and J. Potts, unpublished data), are much smaller in long-winged than in short-winged crickets, at least during the first week of adulthood (Fig. 2; Zera and Mole 1994). Thus, relative to short-winged females, long-winged females not only have a greater proportion of body mass composed of metabolically active flight muscle, but they also have a lower proportion of body mass composed of metabolically inactive ovarian tissue. Preliminary measurements indicate no differences between long-winged and short-winged *G. firmus* in the masses of tissues other than ovaries and flight muscles (A. J. Zera, unpublished data). Finally, Walker et al. (1970) have documented that the basal respiration rate (rate per gram of tissue) of isolated flight muscles of another orthopteran, the desert

locust, is twice as high as that of intact animals or the isolated fat body (a large tissue analogous to the vertebrate liver). This further underscores the disproportionately large contribution of flight muscles to whole-organism basal respiration in flight-capable orthopterans.

The significantly lower respiratory quotient of white relative to pink muscle (Table 5) may partially account for the previously reported reduced lipid and the elevated carbohydrate levels in short-winged (almost exclusively white-muscle) compared with long-winged (primarily pink-muscle) adult *G. firmus* (Zera et al. 1994). The lower respiratory quotient of white muscle suggests an enhanced lipid and diminished carbohydrate use for basal respiration compared with pink muscle. We speculate that this may be an adaptation to conserve lipid flight fuel in long-winged individuals that are capable of flight. *Gryllus* species use lipid as the primary flight fuel, and their capacity for continuous, long-duration flight (at least 10 h in the laboratory) requires a large amount of stored lipid reserves (Zera and Rankin 1989; A. J. Zera, unpublished data).

Fecundity of Flight-Muscle Morphs versus Wing Morphs: Influence on Estimates of the Cost of Flight Capability

An important finding of the present study was the identical elevation in egg production during the first week of adulthood of females with white flight muscle, irrespective of their wing length, compared with females exhibiting pink muscles (all long-winged; Fig. 2). Thus, elevated fecundity is more strongly correlated with flight-muscle phenotype than wing-length phenotype in *G. firmus*. Taking flight muscle into account results in a substantially greater gain in fecundity due to flightlessness (approximately 50% greater gain; Table 6) than does an estimate based on a comparison

between wing-length morphs. To our knowledge, these are the only quantitative data available on the increased fitness gain due to flightlessness resulting from comparisons of flight-muscle morphs and wing-length morphs.

In retrospect, the much stronger correlation between elevated fecundity and reduced flight-muscle mass rather than reduced wing length is not surprising. For example, flight muscles and wings have the same chemical composition on a dry-weight basis, but the absolute reduction in flight-muscle mass is much greater than the reduction in wing mass in flightless individuals (Zera et al. 1994). Thus, a greater amount of nutrients is available for egg production via flight muscle reduction than via wing reduction (Zera et al. 1994; Zera and Denno 1997). Furthermore, previous experiments using artificial wing removal (dealation) to induce flight-muscle histolysis in several cricket species have yielded data similar to those obtained in the present study, which investigated naturally occurring flight-muscle histolysis (Tanaka 1994). Fecundity was higher and age at first oviposition was earlier for dealated females with histolyzed flight muscles than for long-winged females with fully developed muscles. However, these features are equivalent to those of short-winged females with underdeveloped flight muscles (Tanaka 1994). This suggests that the greater gain in fecundity due to reduction in flight muscle rather than wing mass may be a general feature of wing-polymorphic insects.

Results of the present study have important implications for previous and future studies of the evolution of flightlessness. Estimates of the fitness gain due to flightlessness have almost exclusively been assessed in wing-polymorphic insects by comparing the fecundity and age at first oviposition of the short-winged or wingless morph with the long-winged morph (Harrison 1980; Roff 1986; Zera and Denno 1997). If our results for *G. firmus* are general for wing-polymorphic insects, then the fitness gain due to flightlessness has been substantially underestimated, because these studies have not distinguished between long-winged individuals with and without histolyzed flight muscles. Since flight-muscle histolysis is common in the very taxa that have been the most studied with respect to fitness differences between wing morphs (e.g., Orthoptera, Hemiptera, and Coleoptera; Smith 1964; Pener 1985; Roff 1986; Zera and Denno 1997), underestimation of the fitness gain due to flightlessness in these studies seems highly likely. A related, but as yet unexplored, issue concerns the extent to which variation in other physiological characteristics (e.g., lipid and carbohydrate metabolism) is more strongly correlated with variation in flight muscles rather than wing length. The extent to which this occurs will dramatically influence conclusions concerning the costs of flight capability and the mechanisms underlying the trade-off between flight capability and fecundity.

Differences between Underdeveloped and Histolyzed Flight Muscles: Implications for the Evolution of Flightlessness

The evolution of reduced flight muscles can occur by arrested muscle growth in juveniles or by histolysis of grown muscles

in adults. The degree of anatomical and physiological variation between these muscle types and the relative impact of these differences on reproduction are expected to exert a strong influence on which of these evolutionary pathways is favored. Currently, there is no published comparative information on the physiological characteristics of underdeveloped versus histolyzed flight muscles and only minimal anatomical information (e.g., Tanaka 1993, 1994).

In the present study, nearly every whole-thorax physiological feature that we measured (IDH activity, basal respiration) was reduced in underdeveloped white muscles from short-winged crickets compared with histolyzed white muscles from long-winged animals (Tables 3–5). Similarly, white muscles from short-winged crickets were always slightly smaller and exhibited a greater degree of tissue reduction than histolyzed white muscle from long-winged animals of the same age (Fig. 1; Results). However, we observed no association between reduction in physiological or morphological traits (e.g., basal respiration) and elevated reproduction for the two white-muscle types (Fig. 2). This contrasts with the strong association between these features for white and pink muscles (Fig. 2). There are several likely explanations for these results. First, the absolute difference in physiology and anatomy was typically much less between the two white-muscle types than it was between the pink-muscle type and either white-muscle type. For example, the absolute reduction in whole-thorax CO₂ respiration in white muscle from short-winged crickets relative to white, histolyzed muscle from long-winged animals was less than 30% of the reduction between white and pink muscle. The corresponding absolute reduction in whole-thorax CS activity in underdeveloped compared with histolyzed white muscles was less than 2% of the reduction in white compared with pink muscle from long-winged crickets. Thus, if the reduced cost of flight-muscle metabolism elevates egg production, we would expect to see a smaller difference in egg production between the two white-muscle phenotypes than between the pink-muscle phenotype and either white-muscle phenotype. Second, and probably more important, it seems likely that there are other important contributors to enhanced reproduction in flightless females in addition to reduced flight-muscle respiration (e.g., reduced lipid biosynthesis; see *Biochemical and Physiological Characteristics of Flight-Muscle Phenotypes* above). If these additional contributors occur in both types of white-muscled flightless morphs, the effect of variation in flight-muscle features between these morphs on the variation in their egg production would be diminished.

In summary, we have shown that flight-muscle morphs of *G. firmus* differ in many important features of anatomy and physiology. The physiological differences between white and pink muscles provide insight into the nature of the physiological costs of flight capability and the mechanisms underlying the trade-off between flight capability and reproduction. The lack of association between physiological and reproductive variation in females with histolyzed or underdeveloped white

muscles underscores our ignorance of factors other than flight-muscle variation that contribute to reproductive differences between flight-capable and flightless morphs. Finally, the present study underscores the importance of taking flight-muscle variation into account when estimating the fitness gains resulting from flightlessness. Physiological and biochemical studies of wing and flight-muscle morphs are in their infancy. Future studies should provide additional insights into the mechanisms underlying the interaction between flight capability and reproduction and how these physiological interactions are modified during the course of adaptive evolution.

Acknowledgments

Research presented in this article was supported by National Science Foundation grants DEB-9107429 and IBN-9507388 to A.J.Z. K.G. was supported by a National Science Foundation Research Experience for Undergraduates supplement to DEB-9107429. J.S. was supported by a Summer Undergraduate Research Experience Fellowship from a Howard Hughes Grant to the School of Biological Sciences, University of Nebraska.

Literature Cited

- Bergmeyer H.U. 1974. *Methods of Enzymatic Analysis*. Vol. 1. Academic Press, New York.
- Borden J.H. and C.E. Slater 1969. Flight muscle volume change in *Ips confusus* (Coleoptera: Scolytidae). *Can. J. Zool.* 47:29–32.
- Chudakova I.V. and O.M. Bocharova-Messner. 1968. Endocrine regulation of the condition of the wing musculature in the imago of the house cricket (*Acheta domestica*). *Dokl. Akad. Nauk SSSR* 179:489–492.
- Chudakova I.V. and E. Gutmann. 1978. Developmental changes of succinate dehydrogenase, ATPase and acid phosphatase activity in flight muscles of the normal and allatectomized adult cricket, *Acheta domestica* (Orthoptera). *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere* 82:1–15.
- Craig I. 1973. A procedure for the analysis of citrate synthase (E.C. 4.1.3.7) in somatic cell hybrids. *Biochem. Genet.* 9:351–359.
- Dingle H. 1985. Migration. Pp. 375–415 in G.A. Kerkut and L.I. Gilbert, eds. *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Vol. 9. Pergamon, Oxford.
- Dunn O.J. 1959. Confidence intervals for the means of dependent, normally-distributed variables. *J. Am. Statistical Assoc.* 54:613–621.
- El-Ibrashy M.T. 1965. A comparative study of metabolic effects of the corpus allatum in two adult Coleoptera, in relation to diapause. *Mededelingen Landbouwhogeschool Wageningen* 65:1–54.
- Fairbairn D. and D. Roff. 1990. Genetic correlations among traits determining migratory tendency in the sand cricket, *Gryllus firmus*. *Evolution* 44:1787–1795.
- Gomi T., T. Okuda, and S. Tanaka. 1995. Protein synthesis and degradation in the flight muscles of adult crickets (*Gryllus bimaculatus*). *J. Exp. Biol.* 198:1071–1077.
- Harrison R.G. 1980. Dispersal polymorphisms in insects. *Annu. Rev. Ecol. Syst.* 11:95–118.
- Kobayashi M. and H. Ishikawa. 1994a. Involvement of juvenile hormone and ubiquinone-dependent proteolysis in flight muscle breakdown of alate aphid. *J. Insect Physiol.* 40:107–111.
- . 1994b. Mechanism of histolysis in indirect flight muscles of alate aphid (*Acyrtosiphon pisum*). *J. Insect Physiol.* 40:33–38.
- McNab B. 1994. Energy conservation and the evolution of flightlessness in birds. *Am. Nat.* 144:628–642.
- Mole S. and A.J. Zera. 1993. Differential allocation of resources underlies the dispersal-reproduction trade-off in the wing-dimorphic cricket, *Gryllus rubens*. *Oecologia* 93:121–127.
- Pener M.P. 1985. Hormonal effects on flight and migration. Pp. 491–550 in G.A. Kerkut and L.I. Gilbert, eds. *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Vol. 8. Pergamon, Oxford.
- Ready N.E. and R. Najam. 1985. Structural and functional development of cricket wing muscles. *J. Exp. Zool.* 233:35–50.
- Roff D.A. 1986. The evolution of wing dimorphism in insects. *Evolution* 40:1009–1020.
- Shiga S., S. Kogawauchi, K. Yasuyama, and T. Yamaguchi. 1991. Flight behavior and selective degeneration of flight muscles in the adult cricket (*Gryllus bimaculatus*). *J. Exp. Biol.* 155:661–667.
- Smith D.S. 1964. The structure and development of flightless Coleoptera: a light and electronmicroscope study of the wings, thoracic exoskeleton and rudimentary flight musculature. *J. Morphol.* 114:107–183.
- Sokal R.R. and F.J. Rohlf. 1981. *Biometry*. 2d ed. W. H. Freeman, San Francisco.
- Stokes D.R., J.G. Malamud, and D.A. Schreihöfer. 1994. Gender specific developmental transformation of a cockroach bifunctional muscle. *J. Exp. Zool.* 268:364–376.
- Tanaka S. 1986. De-alation, flight muscle histolysis and oocyte development in the striped ground cricket, *Allonemobius fasciatus*. *Physiol. Entomol.* 11:453–458.
- . 1993. Allocation of resources to egg production and flight muscle development in a wing dimorphic cricket, *Modicogryllus confirmatus*. *J. Insect Physiol.* 39:493–498.
- . 1994. Endocrine control of ovarian development and flight muscle histolysis in a wing dimorphic cricket, *Modicogryllus confirmatus*. *J. Insect Physiol.* 40:483–490.
- Veazy J.N., C.A.R. Kay, T.J. Walker, and W.H. Whitcomb. 1976. Seasonal abundance, sex ratio, and macroptery of field crickets in northern Florida. *Ann. Entomol. Soc. Am.* 69:374–380.
- Walker P.R., L. Hill, and E. Bailey. 1970. Feeding activity, respiration and lipid and carbohydrate content of the male desert locust during adult development. *J. Insect Physiol.* 16:1001–1005.

- Weesner F.M. 1960. General Zoological Techniques. Williams & Wilkins, Baltimore.
- Young E.C. 1965. The incidence of flight polymorphism in British Corixidae and the description of the morphs. J. Zool. 146:567–576.
- Zera A.J., C. Borchert, and S. Gaines. 1993. Juvenile hormone degradation in adult wing morphs of the cricket, *Gryllus rubens*. J. Insect Physiol. 39:845–856.
- Zera A.J. and R.F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annu. Rev. Entomol. 42:207–230.
- Zera A.J. and S. Mole. 1994. The physiological costs of flight capability in wing-dimorphic crickets. Res. Popul. Ecol. Kyoto 36:151–156.
- Zera A.J., S. Mole, and K. Rokke. 1994. Lipid, carbohydrate and nitrogen content of long-winged and short-winged *G. firmus*: implications for the physiological cost of flight capability. J. Insect Physiol. 40:1037–1044.
- Zera A.J. and M.A. Rankin. 1989. Wing dimorphism in *Gryllus rubens*: genetic basis of morph determination and fertility differences between morphs. Oecologia 80:249–255.