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# The Physiology of Life-History Trade-offs: Experimental Analysis of a Hormonally Induced Life-History Trade-off in *Gryllus assimilis*

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ABSTRACT: Adult *Gryllus assimilis* given an analog of juvenile hormone exhibited reduced flight muscles and enlarged ovaries similar to those found in naturally occurring flightless individuals of species that are polymorphic for dispersal capability. Control and hormone-treated (flightless) *G. assimilis* did not differ in the amount of food consumed or assimilated on any of three diets that differed in nutrient quantity. Thus, enhanced ovarian growth of flightless individuals resulted from increased allocation of internal nutrients to reproduction (i.e., a trade-off) rather than from increased acquisition of nutrients. Compared with flight-capable controls, flightless *G. assimilis* also had reduced whole-organism respiration, reduced respiration of flight muscles, and reduced lipid and triglyceride (flight fuel) reserves. These differences are remarkably similar to those between naturally occurring flightless and flight-capable morphs of other *Gryllus* species. Results collectively suggest that the increased allocation of nutrients to ovarian growth in flightless *G. assimilis* and other *Gryllus* species results from reduced energetic costs of flight muscle maintenance and/or the biosynthesis or acquisition of lipids. Reduction in these energetic costs appears to be an important driving force in the evolution of flightlessness in insects. Respiratory metabolism associated with flight capability utilizes an increasing proportion of the energy budget of crickets as the quantity of nutrients in the diet is decreased. This leads to a magnification of greater ovarian growth of flightless versus flight-capable individuals on nutrient-poor diets.

*Keywords:* wing polymorphism, life history, trade-off, juvenile hormone, *Gryllus,* crickets.

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Individual life-history traits are often negatively associated with each other. These negative genetic or phenotypic associations are referred to as life-history trade-offs (Roff 1992; Stearns 1992). Examples include increased longevity in lines of *Drosophila melanogaster* selected for delayed reproduction (Rose et al. 1996), reduced overwintering survivorship of lactating (reproductive) females of the red deer, *Cervus elaphus* (Clutton-Brock et al. 1982), and reduced egg size in female *Uta stansburiana* with larger egg clutches (Sinervo and Licht 1991*a*, 1991*b*). Life-history trade-offs play an integral role in life-history evolution because they can constrain the evolution of individual life-history traits (Roff 1992; Stearns 1992).

Starting with Fisher (1930), physiological explanations have been sought for the existence of life-history trade-offs, most commonly in the context of differential allocation of internal resources. For example, negative associations between egg size and egg number or among reproduction, somatic growth, and somatic maintenance are thought to result from a limited internal resource pool that constrains the degree to which nutrients can be optimally allocated simultaneously to several life-history traits (Fisher 1930; Williams 1966; Calow 1981; Pianka 1981; Reekie and Bazzaz 1987; Stearns 1992; Mole and Zera 1993; Zera and Denno 1997).

Despite the pervasiveness of these physiological arguments, detailed functional studies of life-history trade-offs have only recently been undertaken, and many fundamental aspects of this topic remain unresolved or unstudied (Reekie and Bazzaz 1987; Stearns 1989; Sinervo and Licht 1991*a*, 1991*b*; Ketterson and Nolan 1992; Mole and Zera 1993; Zera and Denno 1997). For example, only recently have physiological manipulations provided direct evidence supporting the nutrient constraint model for the trade-off between egg size and egg number in lizards (Sinervo and Licht 1991*a*, 1991*b*). Indeed, the very existence of many postulated trade-offs that result from the differential allocation of internal nutrients has

been questioned (Zera and Denno 1997). Physiological data that strongly support or refute this idea have only recently been obtained for a handful of species (Reekie and Bazzaz 1987; Mole and Zera 1993; Zera and Denno 1997).

A useful experimental system for investigating the physiology of life-history trade-offs is dispersal polymorphism. This polymorphism occurs widely in the Insecta and plays an important role in the life cycle of many species (Harrison 1980; Roff 1986; Zera and Denno 1997). Dispersal polymorphism involves discrete variation in a suite of traits affecting dispersal capability and reproduction. Importantly, flight capability is negatively associated with ovarian growth, leading to morphs that are specialized for reproduction versus dispersal (Harrison 1980; Roff 1986; Zera and Denno 1997). For example, the crickets, *Gryllus firmus* and *Gryllus rubens,* consist of a flight-capable morph that has fully developed wings, fully developed flight muscles, and a high concentration of lipid flight fuel. These species also contain a flightless morph that has underdeveloped and nonfunctional wings and flight muscles and reduced lipid reserves. Flightless females of each species begin oocyte growth significantly earlier than their long-winged counterparts. Recent physiological studies of these two species suggest that this trade-off between early reproduction and flight capability results from the differential allocation of internal resources to ovarian growth versus components of flight capability (i.e., flight muscle maintenance, biosynthesis of lipids; Mole and Zera 1993; Zera and Mole 1994; Zera and Denno 1997; Zera et al. 1997; T. Rooneem and A. J. Zera, unpublished data; see "Discussion" below).

In the present study we used hormonal manipulation to further investigate the physiological basis of the trade-off of resources between flight capability and fecundity in *Gryllus* species. Hormonal manipulation involves the use of exogenous hormones to create a phenotype that resembles a naturally occurring phenotype of the same or different species. The hormonally induced phenotype is then used to test various hypotheses, such as the functional basis of life-history trade-offs (Sinervo and Licht 1991a; Ketterson and Nolan 1992; Ketterson et al. 1996). This technique is increasingly being used to study the mechanistic basis of life-history trade-offs in vertebrates but, prior to the present study, has not been used in analogous evolutionary studies of trade-offs in invertebrates.

Considerable indirect evidence implicates juvenile hormone (JH), a key developmental and gonadotropic hormone, as playing a cardinal role in regulating the trade-off between flight muscles and ovaries in dispersal-polymorphic insects (reviewed in Roff 1986; Nijhout 1994; Zera and Denno 1997). This hypothesis is based on extensive endocrine data from numerous *non*-polymorphic species in which JH positively affects ovarian growth and negatively affects wing and flight muscle growth and development (Nijhout 1994). Furthermore, topical application of juvenile hormone or hormone analogues to flight-capable adults of dispersal-polymorphic species have stimulated the growth of ovaries and the degeneration of flight muscles to levels found in unmanipulated flightless individuals (Tanaka 1994).

In the present study, we constructed an artificial dispersal polymorphism by applying a juvenile hormone analog to produce flightless females of the cricket *G. assimilis,* a species that does not exhibit naturally occurring dispersal polymorphism (Alexander and Walker 1962; Weissman et al. 1980). Adult *G. assimilis* normally emerge with fully developed wings and flight muscles and exhibit delayed reproduction relative to flightless individuals of wing polymorphic species (see "Discussion" below). The present study had the following goals: First, we wished to determine the extent to which the hormonally induced dispersal polymorphism exhibited the same physiological characteristics as naturally occurring dispersal polymorphism. Of particular interest was morph-specific variation in various feeding characteristics (nutritional indices), whole-organism and tissue-specific respiration, and levels of lipid reserves. As mentioned above, our previous studies have identified the energetic costs involved in the maintenance of functional flight muscles and the biosynthesis or assimilation of lipids as being potentially important factors that reduce egg production in the flight-capable morph of dispersal-polymorphic *Gryllus* species (Zera et al. 1994, 1997). Second, we wished to determine the extent to which trade-offs between various physiological components of flight capability and egg production are influenced by the quantity of available nutrients in the diet. Elevated egg production in flightless versus flight-capable morphs of some dispersal-polymorphic insects is magnified under stressful conditions such as nutrient limitation (reviewed in Zera and Denno 1997). However, the physiological basis of this phenomenon has not been investigated. All of our previous trade-off studies were performed using a single condition of unlimited availability of a high-nutrient diet. Finally, we wished to determine the extent to which "third-party traits," that is, organs not directly involved in flight capability or reproduction (e.g., the gut; storage tissue), trade off with ovaries or flight muscles. The influence of these "third party traits" in the trade-off between flight capability and reproduction is potentially important but is a poorly studied phenomenon (Zera and Denno 1997).

## Material and Methods

### *Species and General Rearing Conditions*

*Gryllus assimilis* is a monomorphic fully winged (long-winged) cricket that is widely distributed throughout the West Indies and South and Central American countries bordering on the Caribbean. It has recently been introduced into Florida (Alexander and Walker 1962) and also occurs in the western United States (Weissman et al. 1980). The phylogenetic relationships between *G. assimilis* and other *Gryllus* are currently not well understood. Harrison and Bogdanowicz (1995) recently published a phylogeny of *Gryllus*, based on mitochondrial restriction site data, but the basal branches of the phylogeny are not well resolved.

Crickets used in the present study were taken from a laboratory population that had been initiated with 21 impregnated females collected from Homestead, Florida. The population was founded 2 yr prior to the start of the present study and was maintained by breeding 250–300 individuals per generation (approximately 1.5–2 mo per generation). We never observed the production of short-winged, flightless *G. assimilis* in the laboratory. Nor have short-winged individuals been reported in the field. Except where noted, crickets were reared under the following standard conditions: 16L:8D photoperiod at 28°C at a density of approximately 80 crickets per 10-gallon aquarium during the penultimate stadium and 40–60 crickets per 10-gallon aquarium during the last stadium (last juvenile stage before adult molt). Crickets were fed the dry diet (described in Zera and Rankin 1989) until the last stadium, at which time they were switched to an agar-based diet containing the same components as the dry diet. This agar-based diet is the same as the standard (100%; see below) diet fed to the adults. Last-stadium crickets were checked every 24 h to identify newly molted adults for various experiments.

### *Hormone Treatment*

Adult female *G. assimilis* were treated with the juvenile hormone analogue methoprene. As in many other studies, methoprene was used as a surrogate for juvenile hormone because of its greater potency and longer duration of action (see discussion of Zera and Tiebel [1988] for information and references on this issue). Ten micrograms of methoprene in 2 μL of acetone was chosen as the concentration to be applied. This was based on background studies that documented that this concentration resulted in ovarian growth and flight muscle reduction similar to that observed in the naturally occurring flightless morph of dispersal-polymorphic species (see "Results" and "Discussion" below). Methoprene was applied topically to the abdomen of crickets just posterior to the hind leg and under the raised wings on days 0, 2, 4, and 6 of adulthood (molt to adult = day 0). Full control crickets received neither acetone solvent nor hormone while solvent controls received 2 μL acetone without methoprene. Preliminary analyses demonstrated that individuals fed the 100% diet which received solvent alone (solvent controls) did not differ from full controls for any trait measured (e.g., nutritional indices, lipid levels, etc.). Thus, data from these two types of controls were pooled into a single common control in subsequent analyses.

### *Nutritional Indices*

Growth (GR; absolute mass gain), food consumption (CR), assimilation (approximate digestibility; AD), and conversion of assimilated food into body mass (ECD) were quantified for hormone-treated and control crickets using standard procedures (Waldbauer 1968; van Loon 1989). Formulae used to calculate these nutritional indices are as follows (from Waldbauer 1968). All calculations were performed using dry masses of food, frass, and cricket body mass.

1. Growth (GR) = (Body mass at the end of the feeding trial) − (Initial body mass)
2. Relative growth (RGR) = GR/initial body mass
3. Consumption (CR) = Mass of food ingested during the feeding trial
4. Approximate digestibility (assimilation, AD) = (Mass of food ingested − Mass of frass produced)/Mass of food ingested
5. Efficiency of conversion of digested food to body matter (ECD) = GR/(Weight of food ingested − Weight of frass produced)

Nutritional indices were measured on female *G. assimilis* during the first 7 d of adulthood using the methods described in Mole and Zera (1993, 1994) with slight modification. In the previous studies of Mole and Zera (1993, 1994), mated females were used, while in the present study we used virgin females. This was done because it is difficult both to measure food consumption and frass production of an individual female while keeping that female in the same container with a male for a sufficient period of time to insure mating. Furthermore, mated females require access to oviposition material for egg laying. This further complicates collection of food and frass, which can become mixed with the oviposition material (see Mole and Zera 1993, 1994) for experimental details. Virgin females retain their eggs and thus do

not require access to oviposition material. Ovarian growth is virtually identical for mated and unmated *Gryllus* females during the first week of adulthood (G. Cisper and A. J. Zera, unpublished data). Newly molted (day 0), treated or untreated females were weighed and placed individually in 500 mL containers with a weighed piece of wet diet. On days 2 and 4 the uneaten food and frass produced during the previous 2-d period were removed and stored separately at −20°C and a new piece of weighed food was placed in the container. On day 7 (termination of feeding trial) uneaten food and frass were again removed, and the cricket was weighed and frozen. Crickets used in the experiments, frass, and uneaten food were freeze-dried and their dry weights determined. Dry weights and wet weights, obtained on a separate group of day 0 *G. assimilis,* were analyzed by linear regression and the resulting equation ($r^2 = 0.83$) was used to convert wet weights of day 0 crickets used in the experiment to their corresponding dry weights. Similarly, linear regression equations derived from analyses of wet and dry weights of the experimental diets ($r^2 > 0.98$ for the three diets; see below) were used to convert the wet weight of food eaten by the crickets to dry weight of food eaten.

Nutritional indices were measured on crickets fed one of three diets. The standard (100%) diet was the same as that used in previous feeding experiments on the congeners *Gryllus rubens* and *Gryllus firmus* (Mole and Zera 1993, 1994; Zera and Mole 1994). The 50% and 25% diets were obtained by reducing the dry components of the 100% diet by 50% and 75%, respectively. This was done by adding nonnutritive cellulose such that the total weight of dry components per unit volume of wet diet remained constant for the three diets. Crickets were reared on three diets to determine the extent to which variation in nutritional indices between control and hormone-treated crickets were contingent upon nutrient quantity. Pilot experiments on wing morphs of the congener *G. firmus* documented that all nutritional indices exhibit highly significant variation among the three diets (A. J. Zera, unpublished data).

The same precautions were taken in the present study as were taken in earlier studies (Mole and Zera 1993, 1994) to maximize the accuracy of the nutritional index estimates. Most notably, the same batch of food of a particular diet (e.g., the 100% diet) was given to all control and hormone-treated crickets raised on that diet. Furthermore, care was taken to keep the diet thoroughly wrapped so that it did not lose water during the course of the study. Periodically, wet weight–dry weight relationships for the diets were estimated by linear regression. No significant variation was found in the regression statistics during the course of the experiment for any of the three diets. Pieces of diet were given to crickets such that greater than 50% but less than 100% of the diet was eaten. This was done since errors in nutritional index estimates are inflated when less than 50% of the diet is consumed (Schmidt and Reese 1986; van Loon 1989).

### *Dissections*

Developmental profiles of organ masses were obtained in a parallel experiment in which groups of crickets were raised on each of the three diets used in the feeding studies. On days 0 (same day as molt to adult), 3, 5, or 7, crickets were weighed and the following organs were removed and weighed: ovaries, thoracic flight muscles (plus attached cuticle), gut (contents not removed), and residual (total body mass minus flight muscles, ovaries, and gut plus contents). Control experiments demonstrated that the wet weights of reassembled crickets were consistently greater than 95% of the original whole-cricket weights, thus demonstrating only minimal errors in organ weights due to loss of moisture during the dissection period.

### *Lipid Analyses*

Total lipids and triglycerides were quantified as described in Zera et al. (1994) using the protocol of Bligh and Dyer (1959) with slight modification. Lipids and triglycerides were quantified only in crickets raised on the 100% diet. Briefly, crickets of appropriate ages were individually homogenized in chloroform-methanol containing 0.05% butylated hydroxytoluene. Chloroform-methanol extracts were washed with aqueous potassium chloride to remove nonlipid contaminants (e.g., amino acids and sugars). A small aliquot of the washed extract was used to quantify triglycerides via thin-layer chromatography, and the remainder was evaporated and total lipids were quantified gravimetrically. Following thin-layer chromatography, plates were charred and triglyceride spots were quantified by laser densitometry. Dry weights of crickets were estimated from wet weights using the linear regression equation derived from analysis of wet and dry weights of separate groups of methoprene-treated or control crickets. Regression slopes and intercepts were not statistically different for these two groups and a common regression line, estimated from the pooled data, was used to convert cricket wet weights to dry weights.

### *Respiration Rates and Citrate Synthase (CS) Activities of Flight Muscles and Ovaries*

Respiration rates ($CO_2$ production) and citrate synthase (CS) activities of flight muscles from hormone-treated or

control crickets (100% diet only) were quantified as described in previous studies of *G. firmus* (Zera et al. 1997). Citrate synthase activity is an indicator of mitochondrial volume and aerobic capacity in insect flight muscle (Ready and Najam 1985). Respiration rates were quantified on freshly dissected thoraces (dorso-longitudinal and dorso-ventral muscles with fat body removed) using a Micro-Oxymax respirometer (Columbus Instruments) using the same precautions and background studies described in Zera et al. (1997). Citrate synthase activities were assayed on homogenized dorso-longitudinal and dorso-ventral muscles by the method of Craig (1973).

### *Statistical Analyses*

All statistical analyses were performed using DOS SYSTAT 5.0 (Wilkinson 1990). Growth (absolute mass gain; GR) and consumption (CR) were analyzed by factorial ANOVA with HORMONE (methoprene application vs. control) and DIET (100%, 50%, 25%) as fixed effects. As suggested by Raubenheimer and Simpson (1992), the nutritional indices AD and ECD, which are ratios (see above for formulas), were analyzed by ANCOVA with the numerator of the ratio as the independent variable and the denominator as the covariate. As was the case for GR and CR, HORMONE and DIET were fixed effects. In the case of AD, there was a significant interaction between DIET and COVARIATE, thus precluding an overall ANCOVA. Thus, ANCOVAs of AD were performed separately on data from each of the three diets. Triglyceride and total lipid content were tested via ANCOVA with HORMONE and AGE (days after adult eclosion) as fixed and random effects, respectively, and dry weight as the covariate.

With the exception of ovarian mass, organ masses were analyzed by ANCOVA with total body wet mass as the covariate. The distributions of ovarian masses in the control group and in pooled control and hormone groups were strongly skewed. This was due to the large number of control females with very small ovaries coupled with a few (oldest) females with large ovaries. Distributions could not be normalized by various transformations. Thus, ovarian masses were analyzed as percent total body wet mass using the nonparametric Kruskal-Wallis test. Residual mass (= total body wet mass minus the wet mass of the thorax, ovaries, and gut plus contents) was analyzed by ANOVA. Because ovarian masses were not normally distributed, Spearman's rank correlations rather than Pearson's product-moment correlations were estimated between various organ masses standardized as percent body wet mass.

In cases where various unplanned comparisons of means were performed following the overall ANOVA or ANCOVA (e.g., the nutritional indices, AD and CR) probabilities were adjusted by multiplying them by the number of comparisons performed (Dunn 1959). Adjusted probabilities are denoted as $P^*$. Comparisons between mean ECDs of hormone and control crickets reared on various diets were planned contrasts and thus probabilities were not adjusted. Significance levels for Spearman's correlations between standardized organ masses were based on critical values of table Y of Sokal and Rohlf (1969) for three independent variables estimated from the same data set.

## Results

### *Nutritional Indices*

Means (±SEMs) of the various nutritional indices derived from the feeding trials are presented in figure 1. Results of ANOVAs and ANCOVAs of these data are given in table 1. Hormone-treated (flightless) and control (flight-capable) crickets did not differ in CR (the amount

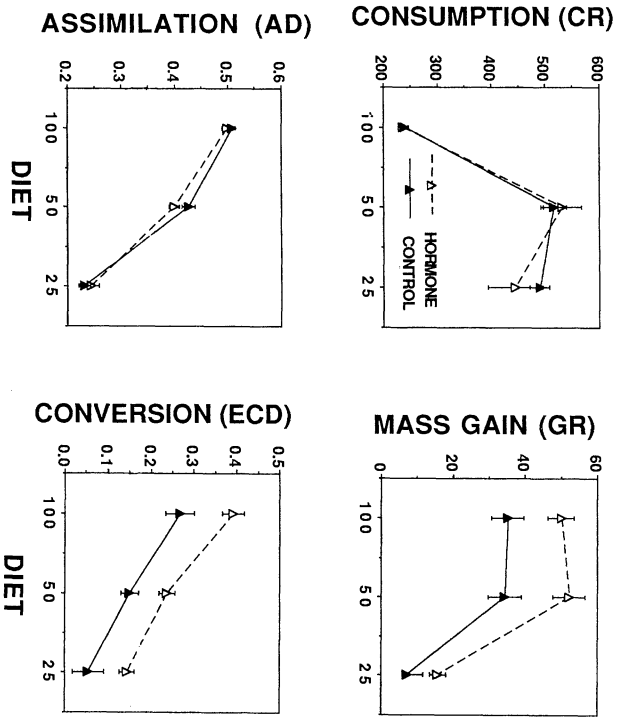


**Figure 1:** Nutritional indices (mean ± SEM) for control and hormone-treated *Gryllus assimilis* on diets of varying nutritional quantity. The various indices and diets are defined in "Methods." Consumption and mass gain are values obtained over the entire 7-d feeding trial and are in milligrams dry mass. Statistical analyses of these data are given in table 1. Sample sizes were as follows: 100% diet, $N = 28$ (hormone) and $N = 24$ (control); 50% diet, $N = 9$ (hormone) and $N = 9$ (control); 25% diet, $N = 9$ (hormone) and $N = 10$ control.

**Table 1:** Results of ANOVAs and ANCOVAs of nutritional indices obtained from hormonally treated and control *Gryllus assimilis*

| | | | | *AD*[a] | | |
|---|---|---|---|---|---|---|
| *Source of variation (df)* | *CR*[b] | *GR* | *ECD* | *100%* | *50%* | *25%* |
| HORMONE (1) | .33 | 10.4** | 14.6*** | .19 | 2.98 | .21 |
| | (1,826) | (3,333) | (1,505) | (23.5) | (1,082) | (34.6) |
| DIET (2) | 131*** | 22.9*** | 22.5*** | ... | ... | ... |
| | (721,943) | (7,326) | (2,328) | | | |
| HORMONE × DIET (2) | .86 | .34 | .41 | ... | ... | ... |
| | (4,742) | (109) | (42.7) | | | |
| Covariate (1)[c] | ... | ... | 13.2*** | 291*** | 47.2*** | 12.1** |
| | | | (1,364) | (36,044) | (17,185) | (1,939) |
| Error | 83 df | 83 df | 82 df | 49 df | 15 df | 16 df |
| | (5,500) | (319) | (103) | (123) | (364) | (161) |

Note: Values are $F$ ratios, and mean squares are in parentheses. $F$ ratios not followed by asterisks did not differ significantly from 0.

[a] Because of a DIET × COVARIATE interaction, ADs were analyzed separately for each diet (see "Methods").

[b] See "Methods" for definitions of nutritional indices.

[c] Covariates were the denominators of the respective nutritional indices (see "Methods").

** $P < .01$.

*** $P < .005$.

of food consumed) during the 7-d feeding trial over all diets or on any of the three diets considered separately. Nor was a significant interaction observed between DIET and HORMONE. However, DIET (the quantity of nutrients per unit mass of diet) had a strong effect on the amount of food consumed by either control or hormone treated *Gryllus assimilis*. Consumption was significantly elevated when nutrients in the standard 100% diet were reduced by 50%, but no further elevation in consumption occurred when nutrients were reduced by an additional 50% (25% diet): Results of ANOVAs: 100% versus 50% diets ($F = 302$, df = 1, 69, $P^* < .001$); 50% versus 25% diets ($F = 2.8$, df = 1, 36, $P^* > .2$). Identical $P$-values for significance tests of main effects and interaction were obtained when CR was analyzed by ANCOVA with initial dry mass as the covariate (results not shown).

As was the case for consumption, AD (approximate digestibility; "assimilation") did not differ between hormone-treated and control crickets fed any of the three diets. As mentioned in "Methods" above, a significant interaction between COVARIATE (dry weight) and DIET precluded an ANCOVA of the entire data set and hence a test for the existence of an interaction between DIET and HORMONE. However, examination of mean ADs for hormone-treated and control crickets on each of the three diets provide no evidence for such an interaction (fig. 1). As is also evident from figure 1, DIET had a strong effect on AD, with mean values decreasing in parallel with reduction in nutrient quantity. Arcsine transformed ADs differed significantly between the 100% and 50% diets ($F = 60.8$, df = 1, 68, $P^* < .0002$) and between the 50% and 25% diets ($F = 213$, df = 1, 26, $P^* < .002$).

In contrast to CR and AD, ECD (the proportion of assimilated food converted into body matter) was significantly higher for hormone-treated crickets compared to controls over all diets (table 1, fig. 1). This was also the case for the 100% and 50% diets and nearly so for the 25% diet when considered individually; ANCOVAs: 100% diet ($F = 8.8$, df = 1, 49, $P < .01$), 50% diet ($F = 8.3$, df = 1, 15, $P < .025$), 25% diet ($F = 4.31$, df = 1, 16, $P < .06$). No interaction was observed between HORMONE and DIET. DIET also had a significant effect on ECD (table 1). Like AD, ECD declined in parallel with nutrient quantity in the diet. Arcsine transformed ECDs (pooled values from hormone-treated and control crickets) were significantly lower in the 50% diet compared with the 100% diet ($F = 12.9$, df = 1, 68, $P^* < .002$) and in the 25% diet compared with the 50% diet ($F = 11.09$, df = 1, 35, $P^* < .005$).

Like ECD, GR (absolute mass gain) was significantly higher for hormone-treated versus control crickets over all diets (fig. 1, table 1). No interaction was observed between DIET and HORMONE. The GR was also significantly higher in hormone-treated compared with control crickets on the 100% diet ($F = 6.5$, df = 1, 50, $P^* < .05$), the 50% diet ($F = 7.77$, df = 1, 18, $P^* < .04$), but not on the 25% diet ($F = 2.9$, df = 1, 17, $P^* > .1$). The

GR also varied with diet. No difference in growth was observed between the 100% and 50% diets ($F$ = 0.0, df = 1, 68, $P^* > .2$) while mass gain was significantly lower on the 25% diet compared with 50% diet ($F$ = 48.9, df = 1, 35, $P^* < .002$). Initial dry mass did not differ between crickets used in the control (mean [SEM] = 157 ± 4.0, $N$ = 43) versus the hormone treatment (155 ± 4.8, $N$ = 47). Thus, relative growth [(final weight − initial weight)/initial weight] varied in an essentially identical manner, as did absolute mass gain. Mean (±SEM) relative mass gain (percent initial dry mass) for treated and control crickets on the various diets were as follows: 100% diet, 34.6 ± 3.1 (hormone) and 23.8 ± 2.9 (control); 50% diet, 36.4 ± 4.0 (hormone) and 23.0 ± 3.9 (control); 25% diet, 10.6 ± 1.6 (hormone) and 4.6 ± 2.6 (control). Sample sizes for these various groups are the same as in figure 1. ANCOVA of relative growth where final dry mass was the independent variable and initial dry mass was the covariate yields identical results ($P$ values for tests of main effects and interaction) as did ANOVA of GR (ANCOVA results not shown).

### *Triglycerides and Total Lipids*

Triglyceride content, quantified in hormone-treated (flightless) or untreated (flight-capable) control crickets that were 0, 2, 4, or 6 d post adult eclosion, are presented in figure 2 as percent dry mass. Total lipids, measured on days 4 and 6, are presented in figure 3 as percent dry mass. Only crickets reared on the 100% diet were subjected to lipid/triglyceride analyses. Methoprene-treated crickets had significantly reduced (40%) triglyceride content relative to control *G. assimilis*. This degree of reduction was observed on the first adult age studied after hormone treatment (day 2) and occurred to a similar extent on the other two adult ages. ANCOVA of these data (covariate = dry mass) resulted in a significant overall effect of HORMONE ($F$ = 26.1, df = 1, 47, $P < .001$) and no significant AGE * HORMONE interaction ($F$ = 0.02, df = 2, 47, $P > .1$). As expected from the similar mean triglyceride content across ages, there was no effect of AGE in the ANCOVA of triglyceride content ($F$ = 1.4, df = 2, 47, $P > .1$). Separate ANCOVAs on each of the three ages yielded a significant effect of HORMONE on day 2 ($F$ = 21.6, df = 1, 14, $P^* < .002$) and day 6 ($F$ = 12.02, df = 1, 15, $P^* < .02$) but no effect on day 4 ($F$ = 5.50, df = 1, 16, $P^* > .05$).

In a similar manner, total lipid content was significantly lower in hormone-treated versus control *G. assimilis* (fig. 3). ANCOVA of lipid content with dry mass as the covariate yielded a significant effect of HORMONE ($F$ = 36.5, df = 1, 32, $P < .001$), AGE ($F$ = 18.3, df = 1, 32, $P < .001$) and no significant HORMONE * AGE interaction ($F$ = 1.02, df = 1, 32, $P > .1$). ANCOVAs performed separately on each age yielded a significant effect of HORMONE on both day 4 ($F$ = 33.8, df = 1, 16, $P^* < .001$) and on day 6 ($F$ = 9.2, df = 1, 15, $P^* < .02$).

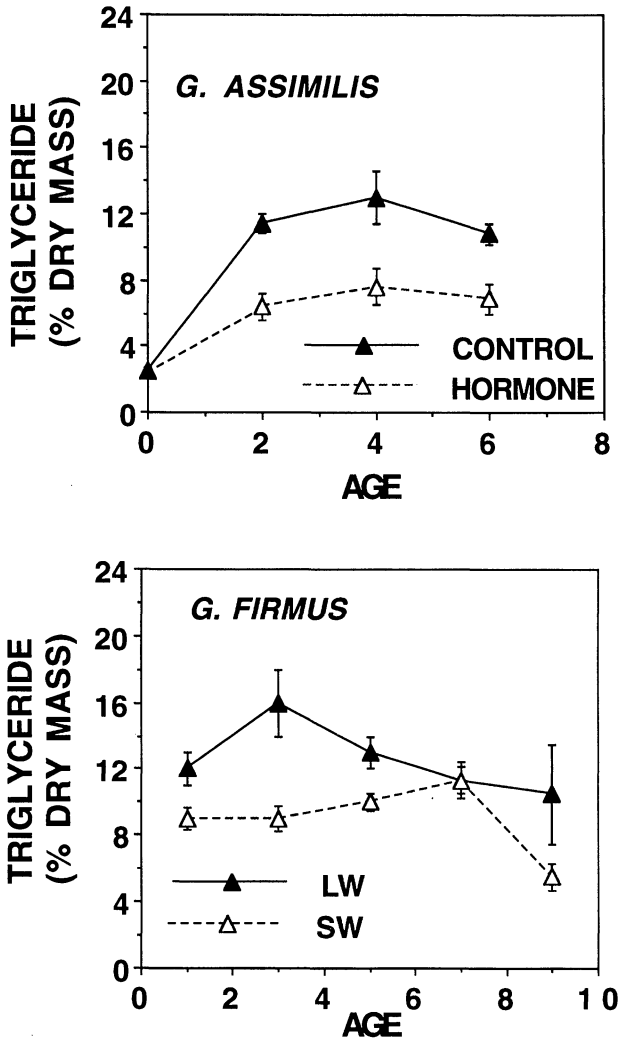


**Figure 2:** Triglyceride content of flight-capable (control or long-wing [LW]) and flightless (hormone [methoprene-treated] or short-winged [SW]) morphs of *Gryllus assimilis* and *Gryllus firmus*. Values are means (±SEM) of 8–10 individuals. Data for *G. firmus* are from Zera et al. (1994). Age is day after molt to adult.

### *Organ Masses*

Ovaries from either control or hormone-treated crickets exhibited a significant increase in mass during the first 7 d of adulthood on each of the three diets (fig. 4, table 2). As expected, methoprene had a strong positive effect on ovarian growth independent of diet. On each day in each diet, ovaries from methoprene-treated crickets were

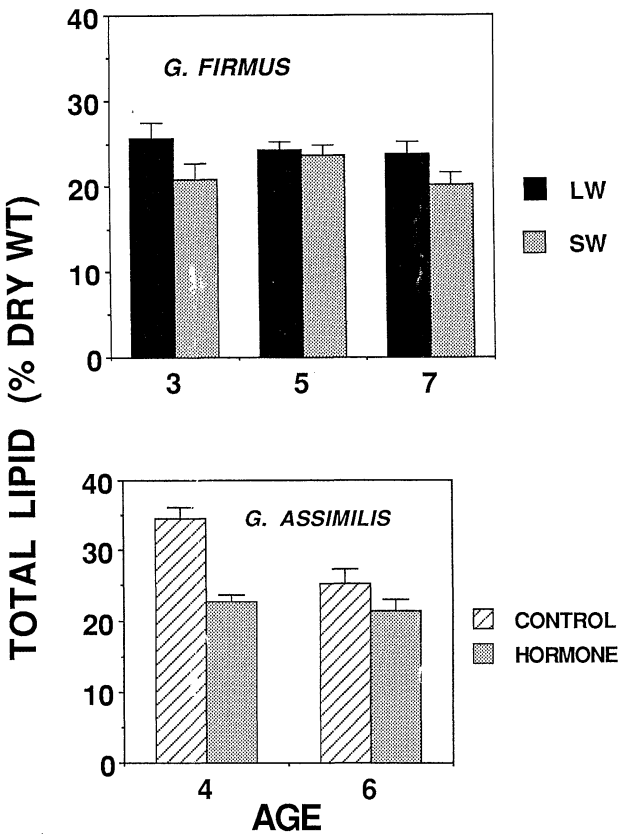


Figure 3: Total lipid content of flight-capable (control or LW) and flightless (hormone [methoprene-treated] or SW) morphs of *Gryllus assimilis* and *Gryllus firmus*. Values are means (±SEM) of 8–10 individuals. Data for *G. firmus* are from Zera et al. (1994). Age is day after molt to adult.

heavier than those from control crickets ($P < .007$ in each of nine Kruskal-Wallis [KW] tests of ovarian mass as % total body wet mass; ovarian masses were analyzed by KW tests because they exhibited a strongly skewed distribution; see "Material and Methods" above). By the end of the experiment (day 7) mean ovarian weight in hormone-treated crickets was three times (100% diet) to five times (25% diet) that of ovaries from control crickets. Diet also exerted a strong effect on ovarian growth (fig. 4). For hormone-treated crickets, significant variation was observed in ovarian mass among the three diets for each age tested ($P < .01$ in each KW test). For control crickets, significant variation was observed on day 7 (KW test; $P < .01$) but not on other days. The absence of variation on days 3 and 5 for control crickets was due to the very slow ovarian growth in these crickets up to and including day 5.

Treatment with methoprene resulted in a significant reduction in size and loss of color of flight muscles (fig. 4, table 2). By day 7, thoracic muscles in hormone-

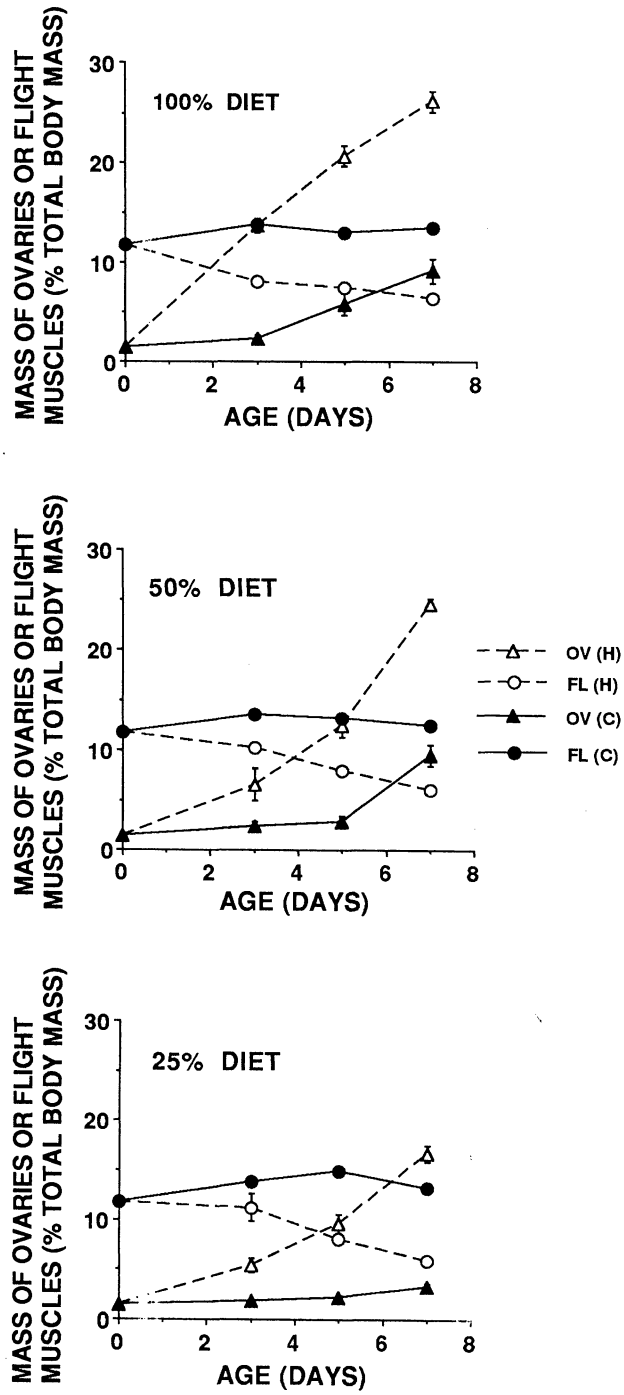


Figure 4: Ovarian and flight muscle mass (% total body wet mass) in hormone-treated and control *Gryllus assimilis* raised on diets of varying nutrient quantity; *OV (H)* and *OV (C)* are ovaries from hormone-treated and control crickets, respectively; *FL (H)* and *FL (C)* are flight muscles from hormone-treated and control crickets, respectively. Values are means (±SEM) of 5–12 (median sample size = 7) individuals. In some cases the error bars are smaller than the symbol and hence are not visible. Age is day after molt to adult.

**Table 2:** Mean (±SEM) ovarian and flight muscle mass (% total body wet mass) between flight-capable (control or LW) and flightless (hormone or SW) phenotypes of three *Gryllus* species

| | *Ovaries* | | *Muscles* | |
|---|---|---|---|---|
| *Species* | *Day 0–1* | *Day 7* | *Day 0–1* | *Day 7* |
| *Gryllus assimilis:*[a] | | | | |
| Control | 1.6 ± .1 | 9.5 ± 1.1 | 11.8 ± .2 | 12.5 ± .4 |
| Hormone | 1.6 ± .1 | 24.5 ± .6 | 11.8 ± .2 | 6.1 ± .2 |
| *Gryllus firmus:*[b] | | | | |
| LW | 1.1 ± .3 | 8.8 ± 1.5 | 10.2 ± .4 | 11.7 ± .4 |
| SW | 1.2 ± .4 | 20.0 ± .3 | 4.8 ± .5 | 6.0 ± .6 |
| *Gryllus rubens:*[c] | | | | |
| LW | 1.1 ± .1 | 19.0 ± 1.3 | 11.5 ± .7 | 13.1 ± 1.3 |
| SW | 1.3 ± .1 | 30.2 ± 2.1 | 6.4 ± .5 | 5.0 ± .5 |

[a] Data are from figure 4 (100% diet); flight muscle from all control crickets was pink (= functional).

[b] Data are from Zera et al. (1997). For LW individuals, flight muscle data are only for individuals with pink, functional muscles (i.e., data for LW individuals with white, histolyzed muscles were not included).

[c] Data are from Mole and Zera (1993). Ovarian and flight muscle masses were measured on 14-d-old adults in contrast to *G. assimilis* and *G. firmus,* which were day 7 adults. Also, LW *G. rubens* were a mixture of pink- and white-muscled individuals.

treated crickets were reduced by 40%–50% relative to control crickets fed the same diet (fig. 4). In addition to being much smaller in size, flight muscles of all hormone-treated crickets were also white on day 3 and on all days thereafter, in contrast to the bright pink color of flight muscles of all control crickets on all days. The loss of color is presumably due to reduced cytochrome content (Zera et al. 1997), which results in a lower capacity for respiration (see below) and renders the crickets flightless. The overall ANCOVA of flight muscle mass yielded a significant two-way interaction between AGE and HORMONE ($F = 16.4$, df = 2, 118, $P < .001$) and a significant three-way interaction between AGE, DIET, and HORMONE ($F = 2.9$, df = 4, 118, $P = .025$). Because of these interactions, the effect of hormone treatment on flight muscle mass was assessed via single-classification ANCOVAs performed separately on each age in each diet treatment. On day 3 and all ages thereafter, hormone-treated crickets had flight muscles that were significantly lighter than those of control crickets ($P < .001$ in each ANCOVA). The single exception was day 3 flight muscles in the 25% diet, which did not differ significantly between hormone-treated and control crickets.

In contrast to the dramatic and consistent variation of ovarian and flight muscle masses between hormone-treated and control crickets across diets and ages, the mass of the gut (plus contents) and residual mass differed to a much lesser degree between hormone-treated and control crickets (fig. 5). The only consistent pattern was a reduction in both gut and residue mass in hormone-treated versus control crickets mainly on the 100% diet and mainly in crickets of increasing age. ANCOVA of gut mass exhibited a significant AGE * DIET interaction ($F = 3.69$, df = 2, 118, $P < .05$) and the only significant main effect was HORMONE ($F = 11.2$, df = 1, 118, $P < .005$). ANOVA of residue only resulted in a significant main effect due to DIET ($F = 11.0$, df = 2, 118, $P < .005$). No other treatments or interactions were statistically significant.

Spearman rank correlations of organ mass (as percent total wet mass) are given in table 3. Flight muscles (thorax) and ovarian masses exhibited a strong negative correlation on each diet. In addition, ovarian mass exhibited a strong negative correlation with gut mass (plus contents) and residue. These correlations became progressively weaker on diets of decreasing nutrient quantity. Finally flight muscle mass was also positively correlated with gut or residue mass, and the strength of these correlations also decreased as nutrient quantity decreased.

### *Respiration Rates and Enzyme Activities*

Rates of $CO_2$ production for flight muscles and ovaries from day 7 control or hormone-treated *G. assimilis* are given in table 4. The $CO_2$ release rates per unit muscle mass (specific respiration rates) were significantly lower in flight muscle from hormone-treated versus control crickets. Differences were even greater when based on

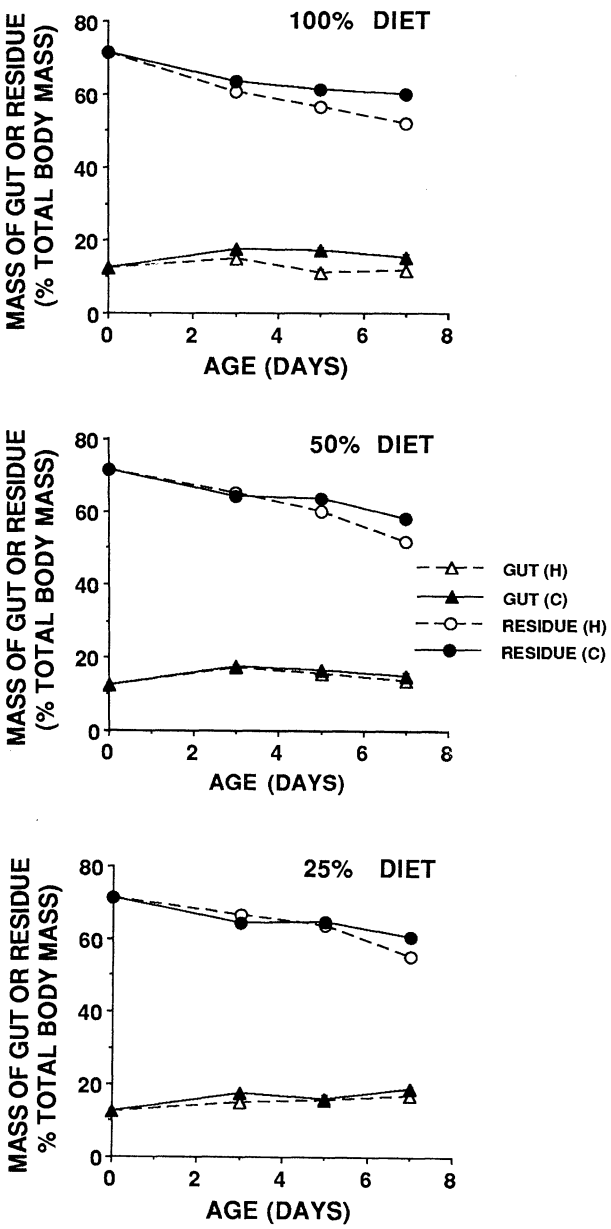


**Figure 5:** Gut (plus contents) and residue mass (% total body wet mass) in hormone-treated and control *Gryllus assimilis* raised on diets of varying nutrient quantity. Residue is total wet mass of a cricket minus wet masses of ovaries, flight muscles, and gut plus contents. Values are means (±SEM) of 5–12 (median sample size = 7) individuals. In most cases the error bars are smaller than the symbol and hence are not visible. Age is day after molt to adult.

**Table 3:** Spearman rank correlations between masses of various organs from *Gryllus assimilis* according to diet

| *Organ* | *Ovaries* | *Gut* | *Residue* |
|---|---|---|---|
| Gut: | | | |
| 100% | −.803** | ... | ... |
| 50% | −.491* | ... | ... |
| 25% | −.215 NS | ... | ... |
| Residue: | | | |
| 100% | −.891** | .553** | ... |
| 50% | −.835** | .235 NS | ... |
| 25% | −.417 NS | −.524** | ... |
| Thorax: | | | |
| 100% | −.893** | .553** | .772** |
| 50% | −.797** | .211 NS | .560** |
| 25% | −.819** | .067 NS | .343 NS |

Note: Correlations were performed on organ masses as percent body wet mass from control and methoprene-treated crickets that were 3, 5, or 7 d post adult eclosion and that were fed the 25%, 50%, or 100% diets. Mean organ masses are presented in figures 4 and 5. Sample sizes were 49, 47, and 41 individuals from the 100%, 50%, and 25% diets, respectively. Correlations were positive unless otherwise noted. NS = nonsignificant ($P > .1$); probabilities were corrected for the three independent variables within each diet treatment using table Y from Sokal and Rolf (1969).

* $P < .05$.

** $P < .01$.

rate per entire thorax. In contrast, $CO_2$ specific respiration rates for ovaries did not differ between hormone-treated and control crickets, while total ovarian respiration rates were higher for the larger ovaries from hormone-treated crickets. Combined respiration rate for ovaries and flight muscles was 79% higher for control *G. assimilis* (2,264 ± 254 nL $min^{-1}$, $n = 6$) compared with hormone-treated females (1,242 ± 167 nL $min^{-1}$, $n = 6$) even though the mass of ovaries and flight muscles comprised a *smaller* proportion of total body mass in control (23%) compared with hormone-treated individuals (32%). Finally, citrate synthase specific and total-thoracic activities (table 5) were significantly lower in flight muscles from hormone-treated versus control crickets (Kruskal-Wallis tests: $P < .01$ in each case).

## Discussion

### *Physiological Differences between the Normal and Hormonally Induced Flightless Morph of* Gryllus assimilis

While a few earlier studies have investigated the proximate role of JH in regulating morphological aspects of dispersal polymorphism (Tanaka 1994), no prior manip-

**Table 4:** Respiration rates for flight muscles and ovaries from flight-capable and flightless morphs of *Gryllus assimilis* and *Gryllus firmus*

| | *Flight muscles* | | *Pair of ovaries* | |
|---|---|---|---|---|
| *Species/measurement* | *Control*[a] | *Hormone*[a] | *Control* | *Hormone* |
| *Gryllus assimilis:* | | | | |
| Per milligram tissue | 32 ± 7[b] | 16 ± 1 | 3.5 ± .3 | 3.4 ± .2 |
| Entire thorax or pair of ovaries | 2,081 ± 434 | 664 ± 32 | 169 ± 11 | 572 ± 23 |
| | *LW*[a] | *SW*[a] | | |
| *Gryllus firmus:* | | | | |
| Per milligram tissue | 29 ± 3 | 19 ± 3 | | |
| Entire thorax | 1,909 ± 239 | 552 ± 36 | | |

Note: Respiration rates for thoracic muscles differed significantly between control and hormone-treated *G. assimilis* ($P < .05$ and $P < .025$ for specific and whole-organ rates, respectively) and between LW and SW *G. firmus* ($P < .05$ and $P < .025$ for specific and whole-organ rates, respectively) as determined by Kruskal-Wallis tests. Specific respiration rates did not differ between pairs of ovaries from control versus hormone-treated *G. assimilis* (K-W test) while whole-ovarian respiration rates differed significantly (K-W test; $P < .001$).

[a] Control or long-winged (LW) crickets have pink, functional flight muscles, while hormone-treated or short-winged (SW) crickets have histolyzed or undeveloped flight muscles, which are nonfunctional.

[b] Mean respiration rates (nL $CO_2$ released $min^{-1}$) were based on independent measures of six single thoraces or six pairs of ovaries from control or hormone-treated *G. assimilis,* eight individual thoraces from LW *G. firmus* or six pairs of thoraces from SW *G. firmus* (rate divided by 2). Data for *G. firmus* are from Zera et al. (1997).

ulations have been undertaken to investigate energetic aspects of this trade-off in dispersal-polymorphic insects, especially in an evolutionary context. In the present study, flight capable (control) and flightless (hormone-treated) *Gryllus assimilis* differed in a suite of physiological and morphological features to the same degree as do naturally occurring flight-capable and flightless morphs of *Gryllus firmus* and *Gryllus rubens.* Moreover, these physiological traits have been identified as potentially key components of the trade-off between flight capability and reproduction (Zera et al. 1994, 1997; Zera and Denno 1997). Results obtained for the hormonally induced polymorphism in *G. assimilis* thus corroborate our previous findings on the existence and functional significance of

**Table 5:** Citrate synthase activities in flight muscle from flight-capable (control or long-winged) or flightless (hormone-treated or short-winged) morphs of *Gryllus assimilis* or *Gryllus firmus*

| *Species/measurement* | *Flight-capable morph* | *Flightless morph* |
|---|---|---|
| *Gryllus assimilis:* | | |
| Per milligram flight muscle | 119 ± 21 | 17.2 ± 2.1 |
| Entire thoracic musculature | 8,568 ± 756 | 672 ± 57 |
| *Gryllus firmus:* | | |
| Per milligram flight muscle | 102 ± 19 | 15.6 ± 4.9 |
| Entire dorso-longitudinal muscles | 1,530 ± 437 | 22.0 ± 9.1 |

Note: Values are means (±SEM) based on six individuals (*G. assimilis*) or three to four individuals (*G. firmus*) and are in units of nmol/min/mg muscle or nmol/min (entire thoracic musculature or entire dorso-longitudinal flight muscles). Note that activities in *G. firmus* were measured exclusively in the dorso-longitudinal muscle while those in *G. assimilis* were measured in the entire thoracic musculature (dorso-longitudinal plus dorso-ventral muscles). Data for *G. firmus* are from Zera et al. (1997).

**Table 6:** Nutritional indices for flight-capable and flightless *Gryllus assimilis, Gryllus firmus,* and *Gryllus rubens*

| | *Nutritional index* | | | | |
|---|---|---|---|---|---|
| *Species* | *ECD* | *AD* | *CR* | *GR (%)* | *RGR (%)* |
| *Gryllus assimilis:* | | | | | |
| Control | .27 ± .03 | .51 ± .01 | 239 ± 7.9 | 36 ± 5 | 24 ± 3 |
| Hormone | .39 ± .03 | .50 ± .01 | 236 ± 10 | 50 ± 4 | 35 ± 3 |
| AN(C)OVA | ** | NS | NS | * | * |
| *Gryllus rubens:* | | | | | |
| LW | .05 ± .01 | .68 ± .02 | 1,080 ± 73 | 36 ± 5 | 27 ± 4 |
| SW | .09 ± .01 | .70 ± .02 | 1,057 ± 58 | 52 ± 4 | 40 ± 3 |
| AN(C)OVA | *** | NS | NS | *** | ** |
| *Gryllus firmus:* | | | | | |
| LW | .11 ± .01 | .67 ± .02 | 981 ± 42 | 64 ± 4 | 45 ± 3 |
| SW | .22 ± .05 | .59 ± .04 | 730 ± 54 | 62 ± 4 | 49 ± 4 |
| AN(C)OVA | ** | * | *** | NS | NS |

Note: All SW (short-winged) and hormone-treated crickets are obligately flightless; all LW (long-winged) individuals initially have a fully developed flight apparatus (Zera et al. 1997, unpublished data). Nutritional indices are defined in "Methods." Sample sizes were as follows: *G. assimilis:* 28 (hormone) and 24 (control); *G. rubens:* 15 (LW) and 27 (SW); *G. firmus:* 28 (LW) and 25 (SW). Nutritional indices were measured during the first 7 d of adulthood for *G. assimilis* (only data from the 100% diet are given here) and during the first 14 d of adulthood for *G. rubens* and *G. firmus*. Data for *G. assimilis* are from figure 1. Data for *G. rubens* are from Mole and Zera (1993) and for *G. firmus* are from Zera and Mole (1994). NS = nonsignificant. Note that the original ECD values for LW and SW *G. firmus* reported in Mole and Zera (1994) are incorrect. The corrected values reported in the present study are from Zera and Mole (1994).

* $P < .05$.

** $P < .025$.

*** $P < .005$.

physiological differences between naturally occurring flight-capable and flightless morphs of *Gryllus* species.

One of the most important findings of the present study was the higher ECD in the hormonally induced, flightless phenotype compared with flight-capable (control) *G. assimilis* (fig. 1, table 1). The ECD is a measure of the proportion of assimilated nutrients that are converted into biomass and is used as a reciprocal measure of respiration (Waldbauer 1968; Slansky and Scriber 1985; van Loon 1989). The logic behind this argument is that increased respiration results in a greater loss of assimilated nutrients as expired $CO_2$, thus resulting in a lower biomass gain per unit food ingested and assimilated. The higher ECD for the flightless phenotype of *G. assimilis,* compared with its flight-capable counterpart, is similar to the elevated ECD of naturally occurring flightless (SW) compared with flight-capable (LW) morphs in both *G. rubens* and *G. firmus* (Mole and Zera 1993; Zera and Mole 1994; Zera and Denno 1997; table 6). We have previously argued that this elevated ECD exists in flightless (SW) *G. firmus* and *G. rubens* because, in contrast to LW individuals, SW crickets are *not* required to allocate assimilated nutrients to respiratory processes necessary for flight capability (e.g., basal respiration of flight muscles, flight fuel accumulation; see below). This results in elevated early fecundity in SW flightless females, which is a general feature of dispersal polymorphism in insects (Zera and Denno 1997). The increase in ECD when flight-capable *G. assimilis* were converted into a flightless phenotype via hormonal manipulation provides strong experimental support for this hypothesis. Recently, similar arguments of energy economy (i.e., reduction in whole-organism respiratory maintenance) have been put forward to explain both the evolution of flightlessness in birds (McNab 1994) and the atrophy of unused organs in numerous vertebrate species (Piersma and Lindstrom 1997).

Previous studies have suggested two potential causes for the elevated whole-cricket respiration of the flightless morph in dispersal polymorphic *Gryllus* species: maintenance of the large, functional flight muscles that comprise 10%–14% of total biomass (basal metabolism; protein turnover) and the biosynthesis or assimilation of triglyceride flight fuels (Mole and Zera 1993; Zera and

Mole 1994; Zera et al. 1994, 1997). Respiration rates of isolated thoraces from LW *G. firmus* with functional flight muscles were three- to fourfold higher than those of SW, flightless females of this species (table 4), and activity of the mitochondrial enzyme citrate synthase was elevated to an even greater degree (70-fold) in the LW morph (Zera et al. 1997; table 5). Concentrations of total lipid and triglycerides (major flight fuel in *Gryllus;* A. J. Zera, J. Sall, and K. Otto, unpublished data) were higher in LW compared with SW *G. firmus* (Zera et al. 1994; fig. 3).

The second important finding of the present study was that the hormonally induced, flightless phenotype of *G. assimilis* strongly resembles the SW morph of *G. firmus* in key physiological features such as reduced flight muscle respiration rate, reduced CS activity, and reduced total lipid and triglyceride content (tables 4 and 5; figs. 2 and 3). Moreover, as in *G. firmus,* this reduction in flight muscle respiration in flightless *G. assimilis* was due not only to the lower specific (mass-adjusted) respiration or enzyme activity but also to the reduced mass of the functional flight muscles in the flightless phenotype. These experimental results are consistent with the notion that the reduced respiration of the flightless morph, which appears to allow additional assimilated nutrients to be allocated to ovarian growth, is due to a reduction in flight muscle maintenance and/or a reduction in energetic processes involved in the accumulation of lipid. At present, it is not possible to determine the relative contribution of these two processes to the elevated respiration of the flight-capable morph. Furthermore, we have yet to directly verify that the increased lipid and triglyceride concentration in the flight-capable morph results from increased biosynthesis as opposed to decreased utilization. Finally, we emphasize that other factors, which have not yet been investigated (e.g., variation in activity levels), may also contribute to differences in whole-organism respiration between flightless and flight-capable morphs.

The present study provides the first estimates of ovarian respiration for flightless and flight-capable morphs of either naturally occurring or hormonally induced dispersal polymorphism. Specific respiration rates are substantially lower (about five- to tenfold) for ovaries compared with flight muscles of *G. assimilis* but do not vary between flight-capable (control) and flightless (hormone-treated) phenotypes (table 4). Even though the ovaries are much larger in flightless compared with flight-capable *G. assimilis,* the elevation in ovarian respiration (about 400 nL $CO_2$ $min^{-1}$) is much less than the decrease in flight muscle respiration in flightless individuals (about 1,400 nL $CO_2$ $min^{-1}$; table 4; also see "Results" above). Thus, the elevated whole-organism respiration of flight-capable versus flightless *G. assimilis* (and probably other *Gryllus* species) likely results from a combination of factors: larger flight muscles that have a high respiration rate and smaller ovaries that have a low respiration rate.

### *Dietary Effects on Nutritional Indices, Reaction Norm of the Trade-off and "Third-Party" Trait Interactions*

Previous studies of the trade-off between flight capability and fecundity were performed exclusively on the standard (100%) diet (Mole and Zera 1993; Zera and Mole 1994), and the degree to which morph-specific differences in nutritional indices and organ masses are contingent upon this specific diet was unknown. Furthermore, trade-offs between flight muscles or ovaries and organs not directly involved in flight or reproduction (i.e., "third party" traits such as the gut) were not investigated (Mole and Zera 1993; Zera and Mole 1994). The present study extends previous physiological investigations of the trade-off between flight capability and fecundity by investigating each of these issues.

Nutritional indices varied across diets (fig. 1; table 1) in a manner similar to variation reported in other insects (Slansky and Scriber 1985; Simpson and Simpson 1989). Consumption increased on the 50% relative to the 100% diet and compensated for decreased nutrient quantity resulting in no reduction in growth. An additional increase in consumption did not occur on the 25% diet, probably due to a constraint on the amount of food that could be consumed and processed. This resulted in a substantial reduction in growth on that diet. The decreased AD on the 50% and 25% diets is probably due in large part to the simple fact that indigestible cellulose is in higher concentration in these diets relative to the 100% diet.

Despite the dramatic variation in CR, AD, GR, and ECD among diets or hormone treatments, these indices either did not differ between morphs on any diet (CR and AD, fig. 1) or differed to an equivalent degree between morphs on all diets (ECD and GR; table 1, also see fig. 1). Thus, no HORMONE × DIET interactions were observed in the ANOVAs or ANCOVAs. The absence of differences in consumption or assimilation between hormone-treated versus control *G. assimilis* on any diet provides strong support for the idea that enhanced ovarian growth of hormone-treated individuals is due to increased allocation of internal resources (i.e., a trade-off) rather than differential nutrient intake or assimilation on each diet. As pointed out previously (Mole and Zera 1993; Zera and Denno 1997; also see van Noordwijk and de Jong 1986), information on consumption and assimilation (= resource acquisition) is essential to determine whether life-history trade-offs result from a trade-off of internal resources (differential allocation) as opposed to some other factor such as the differential acquisition of

external resources. However, some recent studies of trade-offs have provided no information on nutrient assimilation and/or consumption (e.g., Tanaka 1993; Djawdan et al. 1996). The equivalent consumption and assimilation of flight-capable and flightless *G. assimilis* (fig. 1) are similar to our earlier finding that flight-capable (LW) and flightless (SW) *G. rubens* consumed and assimilated an equivalent amount of nutrients on the 100% diet (only diet tested; Mole and Zera 1993; table 6). On the other hand, morphs of *G. firmus* (Mole and Zera 1994; table 6), and *Modicogryllus confirmatus* (Tanaka, 1993) differ in consumption. Thus, in contrast to the consistent elevation of ECD in flightless versus flight-capable morphs in each of the three *Gryllus* species studied, no consistent pattern has emerged with respect to morph-specific differences or lack thereof for consumption.

The consistently lower ECD and GR for control (flight capable) versus hormone-treated (flightless) *G. assimilis* on each of the three diets (fig. 1) suggests that the elevated respiratory cost associated with flight capability is a general feature of this hormonally induced polymorphism and is independent of a specific diet. A noteworthy finding of our study was the parallel decrease in ECDs for the morphs across diets of decreasing nutritional quantity (i.e., no interaction between HORMONE and DIET for ANCOVA of ECD, table 1; also see fig. 1). This pattern suggests that the respiratory cost associated with flight capability utilizes an increasing proportion of assimilated nutrients as the nutrient quantity of the diet decreases. For example, the percent reduction in mean ECD in control relative to hormone-treated *G. assimilis* was 29% on the 100% diet, 34% on the 50% diet, and 71% on the 25% diet. Thus, an increasing proportion of assimilated nutrients appears to be lost as respired $CO_2$ in flight-capable versus flightless *G. assimilis* on diets of decreasing nutritional quantity. Data on organ masses suggest that the proportionally increased respiration in flight-capable versus flightless *G. assimilis* on diets of lower nutrient quantity might result from a constant cost of flight muscle maintenance on all diets. Flight muscle mass is equivalent in flight-capable individuals of the same age raised on different diets (fig. 4). If muscle mass is the main determinant of maintenance costs of flight muscles (basal respiration), then this maintenance cost is expected to be equivalent on all diets. This, in turn, will result in muscle respiration constituting an increasing proportion of the total energy budget on diets of lower nutrient quantity. We plan to test this hypothesis more directly by measuring respiration rates of flight muscles and whole crickets raised on diets of different nutrient quantity.

The flight-capable morph also exhibited an increasingly greater reduction in ovarian mass on the 25% versus 100% diet relative to the flightless morph (fig. 4). For example, on day 7, ovarian mass (as percent body wet mass) in the 25% versus the 100% diet was reduced almost twice as much in flight-capable *G. assimilis* (63%) as it was in flightless individuals of this species (38%). Similarly, on day 5, ovarian mass were reduced by 67% (flight-capable morph) as opposed to 47% (flightless morph) on these diets. This increased differential in ovarian mass between morphs on the 25% versus the 100% diets is consistent with a greater proportion of nutrients being utilized for respiratory maintenance in the flight-capable morph on diets of reduced nutrient quantity which, in turn, results in fewer nutrients available for egg production. An increased fecundity differential between flight-capable and flightless morphs under nutritional stress has been reported for several other dispersal-polymorphic insects (reviewed in Zera and Denno 1997). Thus, data on morph-specific variation in ECD, ovarian mass and flight muscle mass in *G. assimilis* raised on diets of differing nutritional quantity paint a consistent picture of increasing allocation of nutrients to flight capability and decreasing allocation of nutrients to ovarian growth on diets of decreasing nutrient quantity. Despite the consistency of these results, we emphasize that we cannot as yet rigorously assess the statistical significance of these morph-specific differences in ECD, flight muscle mass, and ovarian mass across diets since the entire study was not replicated and hence we have no standard errors for these values. We are currently undertaking a more extensive, replicated study using LW- and SW-selected lines of *G. firmus* to adequately address this issue. Preliminary results from this study are consistent with the results presented here for *G. assimilis* (T. Rooneem and A. J. Zera, unpublished results).

Correlations between the masses of various organs (table 3) and developmental profiles of organ masses (figs. 4 and 5) indicate several cases where trade-offs between organs occur on all diets and other cases where trade-offs are strongly dependent upon a specific diet. As expected, a highly significant negative correlation was found between ovarian and flight muscle mass on each diet, indicating a general trade-off between these two organs. Also of significance were (1) the strong negative correlations between ovarian and gut mass plus contents or mass of the residue and (2) the strong *positive* correlations between flight muscle mass and gut or residue mass. This is the first documentation of "third party" trade-offs between either ovaries or flight muscles and organs not directly involved in flight or reproduction in dispersal-polymorphic insects. Of importance, the negative correlations between gut or residual body mass with ovarian mass were the strongest on the 100% diet. One possible

explanation for these diet-contingent trade-offs is space limitation within the organism. Thus, the substantially larger ovaries in the hormone-treated *G. assimilis* probably compress the gut and restrict the amount of food that can be contained within this organ in addition to reducing the space available for other organs (e.g., fat body) within the thorax and abdomen. The existence of a negative correlation of the greatest magnitude on the 100% diet is consistent with this hypothesis since the ovaries are the largest in crickets fed this diet (fig. 4). The positive correlations between residue or gut mass and flight muscles is possibly an indirect effect of the smaller ovaries found in individuals with larger flight muscles.

The strong negative correlation between ovarian mass and gut mass in *G. assimilis* provides a functional explanation for the reduced consumption of SW versus LW *G. firmus* observed in an earlier study (Mole and Zera 1994). That study was performed on the 100% diet over a 2-wk period, in contrast to the present study which lasted for 1 wk. The reduced consumption of SW female *G. firmus* may have resulted from prolonged restriction of the gut by enlarged ovaries. The inhibitory effect of the enlarged ovaries on digestion (and hence reduced consumption) may have been further exacerbated in that study. For unknown reasons, oviposition of mated females was considerably lower than that typically observed for *G. firmus* in other studies. On the other hand, reproductive females of *Gryllus* species, especially SW females with continuous access to oviposition substrate, often have substantially enlarged abdomen due to enlarged ovaries. Thus, a trade-off between egg production and the volume of food that can be processed by the digestive system may be a important trade-off in this group.

### *Evolutionary Endocrinology of Life-History Trade-Offs*

Hormones likely play a pivotal role in regulating life-history trade-offs (Ketterson and Nolan 1992). They are known to regulate many individual life-history traits and typically have pleiotropic effects. However, except for a few pioneering studies in vertebrates (Sinervo and Licht 1991*a*, 1991*b*; Ketterson and Nolan 1992; Ketterson et al. 1996), the endocrine regulation of life-history trade-offs remains virtually unexplored. Furthermore, endocrine data on genetically based life-history trade-offs, which are especially important in understanding the evolution of these negative trait interactions, have yet to be published for any species.

The present study represents the first use of hormonal manipulation to investigate the physiological basis (i.e., the mechanisms underlying resource allocation and acquisition) of a life-history trade-off in an insect. Results from these hormonal manipulations, together with data from related studies on *Gryllus* and other cricket species collectively implicate juvenile hormone (JH) as an important regulator of each of the key physiological and morphological components of this trade-off in adults. These data are consistent with the long-standing hypothesis that JH plays an important functional role in dispersal polymorphism (Southwood 1961; Wigglesworth 1961; Harrison 1980; Roff 1986; Zera and Denno 1997). However, published data that support the role of JH in regulating morph-specific differences in reproduction and flight capability in adults have come exclusively from experiments involving hormonal manipulation. While this technique is a powerful tool for investigating endocrine mechanisms, it also has limitations. For example, exogenous hormones can give misleading results concerning endocrine regulatory mechanisms since they can induce other endocrine factors that are the true in vivo regulators of some trait of interest (see discussion in Zera and Tiebel 1988). Thus it is essential that hormone manipulation studies be undertaken in parallel with direct measures of suspected in vivo endocrine regulators (e.g., hormone titers or receptor affinities), if a goal is to identify the proximate mechanisms regulating some trade-off. We are taking this combined approach and are in the process of measuring adult hemolymph JH titers in genetic stocks of *G. firmus* that have been selected for the long-winged or short-winged morph (G. Cisper and A. J. Zera, unpublished data).

There is currently intense debate concerning the relative merits of phenotypic manipulations versus genetic approaches to the study of life-history evolution. Reznick (1985, 1992) and Rose and colleagues (Leroi et al. 1994; Rose et al. 1996) have argued that only genetic approaches such as artificial selection are appropriate, if one is interested in studying the evolution of life-history trade-offs. Partridge (1992) has countered that environmental manipulations that produce phenotypic trade-offs provide useful information on this topic. Sinervo and Basolo (1996) and Ketterson (see above references) argue that more specific phenotypic manipulations such as those resulting from the alteration of a particular physiological mechanism by exogenous hormones (i.e., "hormonal engineering") represents a powerful experimental approach to the study of life-history evolution. We argue that a combination of these approaches is essential to adequately understand the physiological mechanisms underlying life-history trade-offs. Strong support for a physiological mechanism regulating a trade-off must include information derived from experimental manipulation of that mechanism. On the other hand, as discussed above, data derived solely from hormonal manipulation can give misleading results and therefore must be evaluated in the context of direct information on in vivo en-

docrine variation. Given the complexity of hormonal interactions, unraveling the endocrine regulation of life-history trade-offs will be difficult (Zera and Denno 1997). However, until this is done we will have a poor understanding of the functional basis of life-history trade-offs and life-history correlations in general.

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## Literature Cited

Alexander, R. D., and T. J. Walker. 1962. Two introduced field crickets new to eastern United States (Orthoptera: Gryllidae). Annals of the Entomological Society of America 55:90–94.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911–917.

Calow, P. 1981. Resource utilization and reproduction. Pages 245–270 *in* C. R. Townsend and P. Calow, eds. Physiological ecology: an evolutionary approach to resource use. Blackwell Scientific, Oxford.

Clutton-Brock, T. H., F. E. Guinness, and S. Albon. 1982. Red deer: behavior and ecology of two sexes. University of Chicago Press, Chicago.

Craig, I. 1973. A procedure for the analysis of citrate synthase (E.C. 4.1.3.7) in somatic cell hybrids. Biochemical Genetics 9:351–359.

Djawdan, M., T. T. Sugiyama, L. K. Schlaeger, T. J. Bradley, and M. R. Rose. 1996. Metabolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. Physiological Zoology 69:1176–1195.

Dunn, O. J. 1959. Confidence intervals for the means of dependent, normally-distributed variables. Journal of the American Statistical Association 54:613–621.

Fisher, R. A. 1930. The genetical theory of natural selection. Dover, New York.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annual Review of Ecology and Systematics 11: 95–118.

Harrison, R. G., and S. M. Bogdanowicz. 1995. Mitochondrial DNA phylogeny of North American field crickets: perspectives on the evolution of life cycles, song, and habitat associations. Journal of Evolutionary Biology 8:209–232.

Ketterson, E. D., and V. Nolan, Jr. 1992. Hormones and life histories: an integrative approach. American Naturalist 140:S33–S62.

Ketterson, E., V. Nolan, Jr., M. J. Cawthorn, P. G. Parker, and C. Ziegenfus. 1996. Phenotypic engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. Ibis 138:70–86.

Leroi, A. M., B. K. Sung, and M. R. Rose. 1994. The evolution of phenotypic life-history trade-offs: an experimental study using *Drosophila melanogaster*. American Naturalist 144:661–676.

McNab, B. 1994. Energy conservation and the evolution of flightlessness in birds. American Naturalist 144:628–642.

Mole, S., and A. Zera. 1993. Differential allocation of resources underlies the dispersal-reproduction trade-off in the wing-dimorphic cricket, *Gryllus rubens*. Oecologia (Berlin) 93:121–127.

———. 1994. Differential resource consumption obviates a potential flight-fecundity trade-off in the sand cricket (*Gryllus firmus*). Functional Ecology 8:573–580.

Nijhout, H. F. 1994. Insect hormones. Princeton University Press, Princeton, N.J.

Partridge, L. 1992. Measuring reproductive costs. Trends in Ecology & Evolution 7:99–100.

Pianka, E. R. 1981. Resource acquisition and allocation among animals. Pages 300–314 *in* C. R. Townsend and P. Calow, eds. Physiological ecology: an evolutionary approach to resource use. Blackwell Scientific, Oxford.

Piersma, T., and A. Lindstrom. 1997. Rapid reversible changes in organ size as a component of adaptive evolution. Trends in Ecology & Evolution 12:134–138.

Raubenheimer, D., and S. J. Simpson. 1992. Analysis of covariance: an alternate to nutritional indices. Entomologia Experimentalis et Applicata 62:221–231.

Ready, N. E., and R. Najam. 1985. Structural and functional development of cricket wing muscles. Journal of Experimental Zoology 233:35–50.

Reekie, E. G., and F. A. Bazzaz. 1987. Reproductive effort in plants. I. Carbon allocation to reproduction. American Naturalist 129:876–896.

Reznick, D. 1985. Costs of reproduction: an evaluation of empirical evidence. Oikos 44:257–267.

———. 1992. Measuring the cost of reproduction. Trends in Ecology & Evolution 7:42–45.

Roff, D. A. 1986. The evolution of wing dimorphism in insects. Evolution 40:1009–1020.

———. 1992. The evolution of life histories. Chapman & Hall, New York.

Rose, M. R., T. J. Nusbaum, and A. K. Chippendale. 1996. Laboratory evolution: the experimental wonderland and the Cheshire cat syndrome. Pages 221–241 *in* M. R. Rose and G. V. Lauder, eds. Adaptation. Academic Press, San Diego.

Schmidt, D. J., and J. C. Reese. 1986. Sources of error in nutritional index studies of insects on artificial diet. Journal of Insect Physiology 32:193–198.

Simpson, S. J., and C. L. Simpson. 1989. The mechanisms of nutritional compensation by photophagous insects. Pages 112–160 *in* E. Bernays, ed. Insect plant interactions. Vol. 2. CRC Press, Boca Raton, Fla.

Sinervo, B., and A. L. Basolo. 1996. Testing adaptation using phenotypic manipulations. Pages 149–185 *in* M. R. Rose and G. V. Lauder, eds. Adaptation. Academic Press, San Diego, Calif.

Sinervo, B., and P. Licht. 1991*a*. Hormonal and physiological control of clutch size, egg size and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. Journal of Experimental Zoology 257:252–264.

———. 1991*b*. Proximate constraints on the evolution of egg size, egg number and total clutch mass in lizards. Science (Washington, D.C.) 252:1300–1302.

Slansky, F., Jr., and J. M. Scriber. 1985. Food consumption and utilization. Pages 87–163 *in* G. A. Kerkut and L. I. Gilbert, eds. Comprehensive insect physiology, biochemistry, and pharmacology. Vol. 4. Pergamon, Oxford.

Sokal, R. R., and F. J. Rohlf. 1969. Statistical tables. W. H. Freeman, San Francisco.

Southwood, T. R. E. 1961. A hormonal theory of the mechanism of wing polymorphism in Heteroptera. Proceedings of the Royal Entomological Society, London 36:63–66.

Stearns, S. C. 1989. Trade-offs in life history evolution. Functional Ecology 3:259–268.

———. 1992. The evolution of life histories. Oxford University Press, Oxford.

Tanaka, S. 1993. Allocation of resources to egg production and flight muscle development in a wing dimorphic cricket, *Modicogryllus confirmatus.* Journal of Insect Physiology 39:493–498.

———. 1994. Endocrine control of ovarian development and flight muscle histolysis in a wing dimorphic cricket, *Modicogryllus confirmatus.* Journal of Insect Physiology 40:483–490.

van Loon, J. J. A. 1989. Measuring food utilization in plant-feeding insects: toward a metabolic dynamic approach. Pages 79–124 *in* E. Bernays, ed. Insect plant interactions. Vol. 3. CRC, Boca Raton, Fla.

van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. American Naturalist 128: 137–142.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Advances in Insect Physiology 5: 229–288.

Weissman, D. B., D. C. F. Rentz, R. D. Alexander, and W. Loher. 1980. Field crickets (*Gryllus* and *Acheta*) of California and Baja California, Mexico (Orthoptera: Gryllidae: Gryllinae). Transactions of the American Entomological Society 106:327–356.

Wigglesworth, V. B. 1961. Insect polymorphism—a tentative synthesis. Pages 103–113 *in* J. S. Kennedy, ed. Insect polymorphism. Royal Entomological Society, London.

Wilkinson, L. 1990. SYSTAT: the system for statistics. Version 5.0. Systat, Inc., Evanston, Ill.

Williams, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. American Naturalist 100:687–690.

Zera, A. J., and R. F. Denno. 1997. Physiological and ecological aspects of dispersal polymorphism in insects. Annual Review of Entomology 42:207–230.

Zera, A. J., and S. Mole. 1994. The physiological costs of flight capability in wing-dimorphic crickets. Researches on Population Ecology (Kyoto) 36:151–156.

Zera, A. J., and M. A. Rankin. 1989. Wing dimorphism in *Gryllus rubens:* Genetic basis of morph determination and fertility differences between morphs. Oecologia (Berlin) 80:249–255.

Zera, A. J., and K. C. Tiebel. 1988. Brachypterizing effect of group rearing, juvenile hormone-III, and methoprene on winglength development in the wing-dimorphic cricket, *Gryllus rubens.* Journal of Insect Physiology 34:489–498.

Zera, A. J., S. Mole, and K. Rokke. 1994. Lipid, carbohydrate and nitrogen content of long- and short-winged *Gryllus firmus:* Implications for the physiological cost of flight capability. Journal of Insect Physiology 40: 1037–1044.

Zera, A. J., J. Sall, and K. Grudzinski. 1997. Flight-muscle polymorphism in the cricket *Gryllus firmus:* Muscle characteristics and their influence on the evolution of flightlessness. Physiological Zoology 70:519–529.

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