University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Anthony Zera Publications | Papers in the Biological Sciences

8-28-1999

Evolutionary Endocrinology of Juvenile Hormone Esterase: Functional Relationship with Wing Polymorphism in the Cricket, *Gryllus firmus*

Anthony J. Zera
University of Nebraska - Lincoln, azera1@unl.edu

Yuan Huang
Shaanxi Normal University, Xian, Shaanxi, People's Republic of China

Follow this and additional works at: http://digitalcommons.unl.edu/bioscizera

Part of the Microbiology Commons

Zera, Anthony J. and Huang, Yuan, "Evolutionary Endocrinology of Juvenile Hormone Esterase: Functional Relationship with Wing Polymorphism in the Cricket, *Gryllus firmus*" (1999). *Anthony Zera Publications.* Paper 17.
http://digitalcommons.unl.edu/bioscizera/17

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.

Published in *Evolution* 53:3 (1999), pp. 837–847. Copyright © 1999 The Society for the Study of Evolution; published by Blackwell Publishing. Used by permission. Submitted July 13, 1998; accepted February 2, 1999.

Evolutionary Endocrinology of Juvenile Hormone Esterase: Functional Relationship with Wing Polymorphism in the Cricket, *Gryllus firmus*

Anthony J. Zera[1] and Yuan Huang[1,2]

[1]*School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68588, USA*

[2]*Department of Biology, Shaanxi Normal University, Xian, Shaanxi, People's Republic of China*

Abstract – The existence, nature, and physiological consequences of genetic variation for juvenile hormone esterase (JHE) activity was studied in the wing-polymorphic cricket, *Gryllus firmus*. Hemolymph (blood) JHE activity was sixfold lower in nascent short-winged (SW) females, relative to nascent long-winged (LW) females, during the last juvenile stadium (stage). Morph-associated genetic variation for JHE activity had two causes, variation in loci: (1) regulating whole-organism enzyme activity; and (2) controlling the degree to which JHE is secreted into the blood. Reduced JHE activity in nascent SW-selected individuals was associated with reduced *in vivo* juvenile hormone catabolism. This suggests that variation in JHE activity during juvenile development may have important physiological consequences with respect to the regulation of blood levels of juvenile hormone and consequent specification of wing morph. This is the first definitive demonstration of genetic variation for hormonal metabolism in any insect and a genetic association between hormone metabolism and the subsequent expression of morphological variation (wing morph). However, we have not yet firmly established whether these associations represent causal relationships. In contrast to the clear association between JHE activity and wing morph development, we observed no evidence indicating that variation in JHE activity plays any direct or indirect role in causing the dramatic differences in ovarian growth between adult wing morphs. Variation in JHE activity also does not appear to be important in coordinating the development of wing morph with the subsequent expression of reproductive differences between adult morphs. Finally, genetic variation for the developmental profiles of JHE activity during juvenile and adult stages are remarkably similar in three *Gryllus* species. This suggests that genetic correlations between JHE activities during different periods of development, which underlie these activity profiles, have been conserved since the divergence of the three *Gryllus* species.

Keywords – Crickets, *Gryllus firmus*, JH, JHE, juvenile hormone, juvenile hormone esterase, wing polymorphism.

The evolutionary genetics of hormonal variation is a poorly studied topic in evolutionary biology. At issue are the extent and nature of genetic variation and covariation for various endocrine traits (*e.g.*, hormone titers, receptor characteristics, and activities of hormone-metabolizing enzymes) and the functional relationship between variation in endocrine and whole-organism features. Thus far genetic information on naturally occurring endocrine variation and covariation is almost exclusively limited to a single endocrine trait, the activity of juvenile hormone esterase (JHE), in a few insect species (Zera and Zhang 1995; Gu and Zera 1996; Zera *et al.* 1998; Zera and Denno 1997 and references therein; see below). Similarly, the functional relationship between variation in endocrine and whole-organism traits has been studied in only a few cases (Zera and Tiebel 1989; Zera *et al.* 1989; Fairbairn and Yadlowski 1997; Roff *et al.* 1997; Zera and Denno 1997). This paucity of information limits our understanding of both the microevolution of the endocrine system itself and the mechanisms underlying the evolution of organismal traits that are hormonally regulated (*e.g.*, life-history traits).

The present study is the first in a series in which we attempt to identify specific components of the insect endocrine system that have been altered during the evolution of an ecologically important developmental and life-history polymorphism, wing polymorphism in the cricket *Gryllus firmus* . Wing polymorphism involves discrete variation in a suite of traits affecting dispersal capability and reproduction (Harrison 1980; Roff 1986; Dingle 1996; Zera and Denno 1997). Polymorphic species contain a flight capable morph, with fully developed wings and fully-developed flight muscles at the adult molt (LW), and a flightless morph that exhibits underdeveloped and nonfunctional wings and flight muscles (SW). The flightless morph begins oocyte growth significantly earlier than its long-winged counterpart.

Wing-polymorphism has been extensively discussed with respect to the endocrine mechanisms that regulate ecologically important polyphenism and genetic polymorphism (Southwood 1961; Wigglesworth 1961; Gould 1977; Harrison 1980; Roff 1986; Nijhout 1994; Zera and Denno 1997). The foci of these discussions have been juvenile hormone (JH), a key developmental and reproductive hormone, and, more recently, JHE, an enzyme that degrades and partially regulates the concentration of JH in some insects (Hammock 1985; Roe and Venkatesh 1990). In all juvenile insects studied thus far, including species of *Gryllus,* a high concentration of JH causes retention of juvenile characteristics by causing a molt from one juvenile stage to another (Zera and Tiebel 1988; Zera *et al.* 1989; Nijhout 1994). Metamorphosis ensues during the last juvenile stage, when JH drops to a very low level due to reduced hormone biosynthesis and increased degradation by JHE (Nijhout 1994; de Kort and Granger 1996). In the adult stage, JH regulates many aspects of reproduction such as the synthesis of yolk proteins and their uptake by the eggs (Wyatt and Davey 1996). The classical JH/wing polymorphism hypothesis (Southwood 1961; Wigglesworth 1961; Nijhout 1994; Zera and Denno 1997) posits that individuals destined

to develop into the short-winged (SW) morph have an elevated JH titer (concentration) at some critical period in development compared with individuals destined to become long-winged (LW), flight-capable adults. This elevated titer inhibits the full development of wings and flight muscles. In the adult stage, an elevated JH titer in the SW morph purportedly underlies its earlier egg production.

Despite considerable speculation and discussion, the only direct test of the JH/wing polymorphism hypothesis has been undertaken in the cricket, *Gryllus rubens* (Zera and Tiebel 1989; Zera *et al.* 1989; reviewed in Zera and Denno 1997; Zera 1999). Most studies in this species have focused on the differential degradation of JH by JHE as a key aspect of the endocrine regulation of alternate morph development. JHE activity is substantially elevated in nascent LW versus SW morphs of *G. rubens* during the last juvenile stage (Zera and Tiebel 1989). Furthermore, elevated JHE activity strongly cosegregates with the LW morph in interstock crosses and is associated with elevated JH catabolism (Zera and Holtmeier 1992). These results are consistent with JHE regulating wing morph development by the differential degradation of JH. However, JH titers in nascent morphs are highly variable and a clearly reduced JH titer in the morph with elevated JHE activity is not seen (Zera *et al.* 1989; Zera and Denno 1997; Zera 1999). Thus, although it is clear that JHE activity is strongly correlated with wing morph, it is not clear whether JHE activity has a significant effect on the JH titer or whether functionally significant JH titer variation exists between wing morphs. In addition to these uncertainties, our previous studies in *G. rubens* were limited to only one LW-selected and one SW-selected line that were available at that time. The absence of replicate genetic stocks precludes a complete assessment as to whether differences between wing morphs for most endocrine traits (*e.g.*, JH catabolism in juveniles) resulted from genetic as opposed to environmental variation.

Here we report on a continuation of our studies on the evolutionary genetics of JHE and wing polymorphism. Because of difficulties in the long-term maintenance of selected lines of *G. rubens* we have turned to its congener, *Gryllus firmus,* as an experimental organism. As is the case with *G. rubens, G. firmus* is wing polymorphic in natural populations (Veazy *et al.* 1976). Using artificial selection, we have obtained replicated stocks of *G. firmus* that are genetically differentiated with respect to wing morph (see Results). We are characterizing these stocks with respect to variation in JH degradation by JHE and other enzymes to ascertain their role in the regulation of wing polymorphism. These studies are part of larger project in which we are assessing the role of various hormones and hormonal regulators (*e.g.*, JH, ecdysteroids, JHE) as causative factors in regulating both developmental and reproductive aspects of wing polymorphism.

The main goals of the present study were to determine whether differences in JHE activity and JH catabolism, which were previously documented in juveniles and adults of one pair of LW-selected and SW-selected lines of *G. rubens,* also occur in replicate selected lines of the congener *G. firmus.* Furthermore, we expand on our earlier studies by quantifying whole-organism and tissue distribution of JHE activity. These data provide important information on the nature of loci responsible for genetic variation in hemolymph JHE activity. Finally, we investigated whether the activity of the other key enzyme involved in JH catabolism, JH epoxide hydrolase, also exhibits morph-associated genetic variation, to assess whether this enzyme might influence morph development.

METHODS

Species, Stocks, Rearing Conditions, and Sampling Schedule

Gryllus firmus exists in the southeastern United States as a short-winged, flightless morph or as a long-winged morph, some of which are capable of flight (Alexander 1968; Veazy *et al.* 1976; Zera *et al.* 1997). The congeners, *G. rubens* and *G. assimilis,* (also discussed in this report), similarly occur in the southeastern United States. *Gryllus rubens,* like *G. firmus,* is wing polymorphic, whereas *G. assimilis* is long-wing monomorphic (*i.e.*, contains only long-winged individuals; Alexander 1968; Veazy *et al.* 1976). *Gryllus assimilis* has only recently been identified in the United States, and, presumably, is a recent immigrant (Alexander and Walker 1962). All species are ground-dwelling and exhibit a facultative diapause allowing continuous rearing in the laboratory. The phylogenetic relationships among *Gryllus* species are 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. We have recently constructed a phylogeny of eastern United States and European *Gryllus,* based on DNA sequences of the mitochondria1 cytochrome *b* and 16 S ribosomal RNA genes (M. Southerlin, Y. Huang, G. Orti, D. S. Siegel-Causey, and A. J. Zera, unpubl. data). *Gryllus assimilis, G. firmus,* and *G. rubens* each occur in different clades. However, the relationships among these clades currently are not well resolved.

Gryllus firmus used in the present study originated from genetic stocks selected for the long-winged (LW) or short-winged (SW) morph. Full details of the selection experiment will be reported elsewhere. Briefly, three blocks, each of which contained a LW-selected (L), a SW-selected (S), and a control (C) line, were derived from a single base population. The block to which a particular line belongs is denoted by the number following its letter (*e.g.*, L-1, S-1, and C-1 are, respectively, the LW-selected, SW-selected, and control lines of block-1). The base population had been derived from 30 gravid female *G. firmus* collected in Gainesville, Florida. For the three L lines, only LW males and females were bred; for the three S lines, only SW males and females were bred. For the three C lines, adults were randomly chosen as breeders; 200–250 adults were bred for each line during each generation. In the present study, crickets were used during the fourth to sixth generations of selection. Frequencies of the various wing morphs in the selected lines during these generations are given in Table 1.

In this study, we exclusively focus on the endocrine underpinnings of genetically differentiated wing morphs. We only report comparisons of endocrine traits between LW individuals from the L-lines (LW[L]) and SW individuals from the S-lines (SW[S]). Other types of comparisons such as variation in endocrine traits between LW and SW morphs from the same selected (*e.g.*, LW[L] vs. SW[L]) or control

TABLE 1. Mean (± SEM) frequency of long-winged (LW) females in LW-selected (L) and short-winged selected (S) lines of *Gryllus firmus*. Sample sizes ranged from 70 to 230 individuals per line.

Generation	L-lines	S-lines
4	0.71 ± 0.01[1]	0.29 ± 0.04
5	0.75 ± 0.003	0.32 ± 0.03
6	0.81 ± 0.04	0.34 ± 0.02

[1] Mean (± SEM) frequency of the LW morph in three LW-selected (L) or three SW-selected (S) lines.

line will be reported elsewhere (Zera *et al.*, unpubl.). Note that in the present report when we refer to LW individuals we refer ex-clusively to those from the L-selected lines and when we refer to SW individuals we refer exclusively to those from the S-selected lines. Finally, all endocrine characterizations were performed exclusively on females.

Crickets were reared at 30°C under a 16:8 L:D dark photoregime. Crickets were fed the standard dry diet, described in Zera and Rankin (1989), until the stage before the penultimate one: after which they were fed an agar-based wet diet, which contained the same components as the dry diet. Endocrine characteristics were measured during days 3, 5, and 8 of the nine-day last stage and on days 3 and 7 of adulthood. These days were chosen to obtain endocrine information during the early, mid and late last-stage, which are known to differ dramatically in endocrine features such as JHE activity in the congeners *G. rubens* and *G. assimilis* (Zera and Tiebel 1989; Zera and Zhang 1995) and during adult stages, which differ considerably in ovarian development (see Results).

Enzyme Assays

Hemolymph Juvenile Hormone Esterase Activity—Hemolymph (blood) JHE activities were measured using standard procedures as described in Zera and Zhang (1995 and references therein). Briefly, JHE activities were measured using the radiochemical assay of Hammock and Sparks (1977). Background studies (data not shown) documented the conditions under which enzyme activity was linear with respect to assay time and enzyme (hemolymph) concentration. These studies also documented that JH acid was essentially the sole metabolite (> 95%) produced when JH was incubated with dilute hemolymph. The sole production of JH acid is a prerequisite for use of the Hammock and Sparks (1977) assay to measure JHE activity. Hemolymph (2 μl) was obtained from individual crickets from a small cut on the cerci and was diluted 1/30 with 0.1 M K^+ phosphate buffer (pH 7.1) and kept on ice until assayed (less than one week). No loss in JHE activity takes place during this time. Because none of the lines was pure breeding during this study (Table 1), each cricket from which a blood sample had been taken was reared separately from other crickets until its wing length phenotype could be determined at the adult molt. This allowed us to match JHE activity with the wing morph for each individual.

Whole-Body Juvenile Hormone Esterase and Juvenile Hormone Epoxide Hydrolase Activity—Nonhemolymph tissues in *Gryllus* species contain two JH-degrading enzymes: JHE and JH epoxide hydrolase (Zera and Zhang 1995). The activities of these two enzymes, in whole-cricket homogenates, were measured simultaneously using the radiochemical assay of Share and Roe (1988). Briefly, this method consists of two replicated sets of assays for each sample, one set the JHE inhibitor OTFP (3-octylthio-1,1,1-trifluoro-2-propanone) and the other set not containing this inhibitor. Activity in tubes containing OTFP is due solely to JH epoxide hydrolase, whereas activity in tubes without OTFP is due to both JHE and JH epoxide hydrolase. JHE activity is obtained as the difference between values from these two sets of assays. As in the case of the hemolymph JHE assay, background studies were performed to identify the conditions of homogenate dilution and assay time, which resulted in a linear relationship between enzyme concentration and JHE or JH epoxide hydrolase activity and assay time and concentration of JH acid (product of JHE reaction) and JH diol (product of JH epoxide hydrolase). Assays employing thin-layer chromatography in which each of the metabolites of the JHE and JH epoxide hydrolase reactions were directly quantified gave the same results as those obtained by the assay of Share and Roe (1988).

Individual crickets were weighed and homogenized in a volume (ml) of 0.1 M phosphate buffer (pH 7.1) that was five times their wet mass in grams. Homogenates were centrifuged at 14,000 g for 3 min and the supernatants were assayed immediately for JHE and JH epoxide hydrolase activities. Background studies documented that JH epoxide hydrolase activity begins to decrease within 30 min after homogenization. Enzyme activities were always measured within 30 min. Prior to homogenization, a 2 μl hemolymph sample was taken from each cricket and its JHE activity was determined to identify the nascent wing morph of each juvenile cricket at the time of homogenization. All lines produced LW and SW morphs (Table 1) and these individuals are indistinguishable in external morphology during the last stage. Prior studies indicated that hemolymph JHE activities from LW versus SW crickets from the same line on days 5 or 8 of the last stage exhibit virtually nonoverlapping distributions (*e.g.*, see Fig. 1). Therefore, hemolymph JHE activity can be used for morph identification during the last stage. Homogenized individuals whose hemolymph JHE activities fell within the 95% confidence interval of mean JHE activity of previously assayed LW *G. firmus* were designated as nascent LW individuals. The same was done for nascent SW individuals. JHE activities of 16% of *G. firmus* (41/254 individuals) fell between these two confidence intervals and were excluded from the analyses of whole-cricket JHE and JH epoxide hydrolase activities.

Estimation of Hemolymph Volume and Tissue Distribution of Juvenile Hormone Esterase

To estimate the tissue distribution of JHE activity, specifically the percentage of whole-organism JHE activity that is found in the hemolymph (blood) compartment, we first estimated the total hemolymph volume in a group of crickets that spanned the range of morphs and ages of individuals whose JHE activities were quantified. Hemolymph volume was estimated by the inulin-dilution method as described in Zera and Holtmeier (1992). Hemolymph volumes were measured in five crickets of each of the four ages, two wing morphs, and three blocks from which crickets were derived for JHE

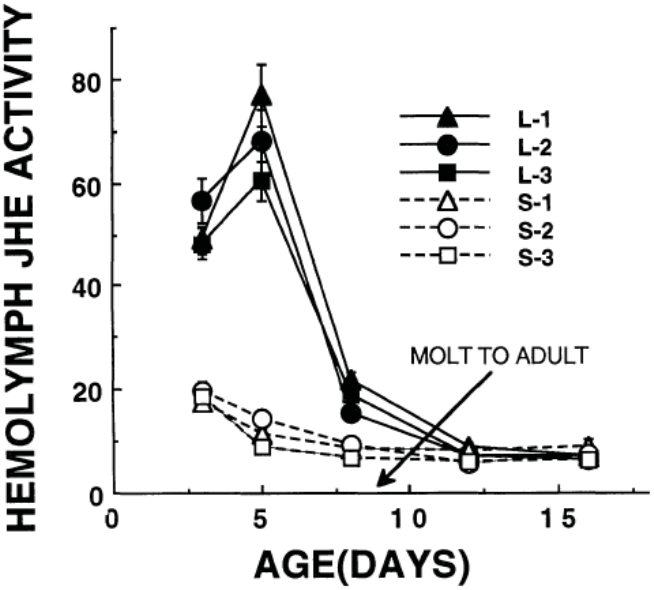

Figure 1. Mean (±SEM) hemolymph juvenile hormone esterase activity in long-winged (LW) females from the LW-selected (L) lines and short-winged (SW) females from the SW-selected (S) lines during the last juvenile stage and first week of adulthood of *Gryllus firmus.* Number after the symbol corresponds to the block to which the line belongs (see Methods). Each symbol represents the mean of 10–38 individual crickets. Day 0 corresponds the first day of the last juvenile stage. Molt to adult occurs on approximately day 9 of the last juvenile stage for both morphs (see Results). Activities are in nmol JH acid/min ml hemolymph.

and JH epoxide hydrolase measurements. ANOVA indicated no significant differences in hemolymph volume (as arcsine-transformed percent wet weight) between wing morphs of the same age for any age or between day-5 and day-8 juveniles (ANOVA). However, slight but significant variation was observed among day-3 adults (hemolymph volume = 17%), day-7 adults (20%), and day-5 or day-8 juveniles (21%). Thus, we used these three values when computing hemolymph volumes from wet weights of crickets of various ages. JHE activity for the entire hemolymph pool of an individual cricket was estimated as the product of its estimated hemolymph volume and its hemolymph JHE activity (measured on 2 μl of hemolymph prior to homogenization; see above).

In vivo Juvenile Hormone Degradation

In vivo JH degradation was quantified as described previously for *G. rubens* and *G. assimilis* (Zera and Holtmeier 1992; Zera and Zhang 1995). Briefly, approximately 250,000 DPM of racemic JH-III (New England Nuclear; 721.56 GBq/mmol; 19.5 Ci/mmol) dissolved in olive oil was injected into the abdominal hemocoel of *G. firmus.* After incubating at 30°C for 50 min, crickets were individually homogenized in ethyl acetate and the amount of undegraded JH was determined by thin-layer chromatography and liquid scintillation spectrometry. Prior to the injection of radiolabeled JH into juveniles, a 2-μl hemolymph sample was taken to determine the hemolymph JHE activity for each cricket and thus its wing length phenotype (see Methods on whole-cricket JHE and JH epoxide hydrolase activities). Previous studies on both *G. rubens* and *G. assimilis* documented that very little (< 3%) radiolabel was excreted during the period of incubation (Zera and Holtmeier 1992; Zera *et al.* 1993; Zera and Zhang 1995) and thus radiolabel excretion was not quantified in the present study.

TABLE 2. Mean (± SEM) ovarian mass (percent total body wet mass) in long-winged (LW) *Gryllus firmus* from the L-selected lines and in short-winged (SW) individuals from the S-selected lines. Sample sizes ranged from eight to 12 individuals per morph per line.

	Age[1]			
	Day 3		Day 7	
Block	LW	SW	LW	SW
1	4.7 ± 0.8	5.7 ± 0.8	14.9 ± 2.2	24.1 ± 1.9
2	3.2 ± 0.5	3.5 ± 0.4	12.4 ± 1.8	21.2 ± 2.4
3	3.4 ± 0.4	6.1 ± 0.9	13.5 ± 2.3	22.5 ± 2.1

[1] Days after eclosion to adulthood.

Statistical Analyses

Genetic differences between L and S lines with respect to wing morph frequencies and between LW and SW morphs with respect to ovarian masses and various endocrine traits were assessed by paired *t*-tests (Sokal and Rohlf 1989). The difference between the mean value for a particular trait for LW individuals from an L line and the mean value for SW individuals from the S line of the same block was computed. This was repeated for the three blocks. The mean and standard error of these differences was then used to compute a *t*-statistic with 2 df (number of blocks minus one). Paired *t*-tests were performed separately on each day of development for each endocrine factor or for ovarian weights. Using mean values as individual variates eliminates the problem of pseudoreplication, which would result if genetic differences for these traits were assessed by an ANOVA in which the degrees of freedom were based on the number of individuals sampled per line (Rose *et al.* 1996). Data consisting of frequencies or proportions, such as wing morph frequencies and ovarian masses (as percent total body wet mass), were arcsine transformed prior to analyses.

Results

Wing Morph Frequencies, Ovarian Masses, and Duration of Development of Wing Morphs

Wing morph frequencies for the L and S lines during the fourth to sixth generations of artificial selection are given in Table 1. These are the same generations during which endocrine traits were compared between wing morphs of these selected lines. The mean frequency of the LW morph averaged over the three L lines was higher than the corresponding frequency for the three S lines during each of generations 4–6 (results of paired *t*-test; $P < 0.01$ in each case). Thus, the L and S lines were genetically differentiated with respect to wing morph. Ovarian masses for LW and SW morphs on days 3 and 7 of adulthood are presented in Table 2. For each block, ovaries were small in both morphs on day 3 (< 7% body wet mass) and did not differ significantly between LW and SW fe-

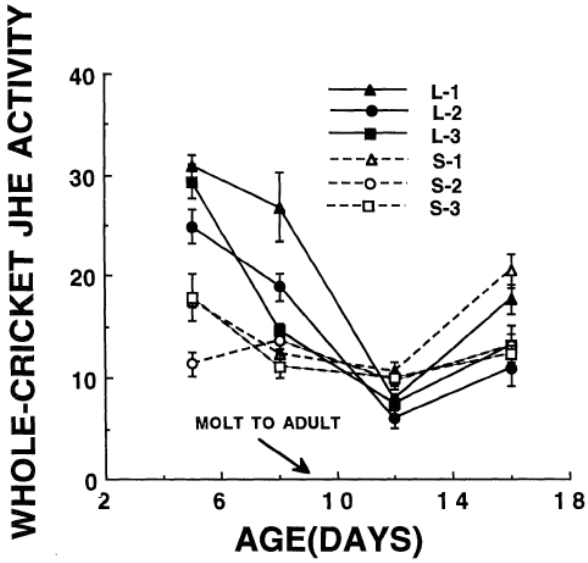

Figure 2. Mean (± SEM) whole-organism juvenile hormone esterase activity in long-winged (LW) females from the LW-selected (L) lines and short-winged (SW) females from the SW-selected (S) lines during the last juvenile stage and first week of adulthood of *Gryllus firmus*. Symbols and ages are the same as those in Figure 1. Each symbol represents the mean of five to 21 individual crickets. Activities are in pmol JH acid/min mg wet weight.

males (t = 1.90, P < 0.05). On day 7, ovaries were substantially larger in SW versus LW females of each block. Means of ovarian masses for SW-selected females were significantly higher than means for LW-selected females (t = 78, P < 0.001). Duration of the last stage, averaged over the three selected lines, was 8.9 ± 0.08 days for LW females and 9.1 ± 0.15 days for SW selected females. These values did not differ significantly (t-test, P > 0.1).

Genetic Differences in Juvenile Hormone Esterase Activity between Wing Morphs

Mean hemolymph JHE activity for LW females from each of the three L-selected lines and SW females from each of the three S-selected lines on each of three days of the last stage and two days of adulthood are presented in Figure 1. In LW females, JHE activity peaked during the middle of the last stage and dropped to much lower levels in adults. In SW individuals, activities were low throughout the last stage and during adulthood. Dramatically higher activity was observed in LW versus SW females for each pair of lines from each of the three blocks on each of the three juvenile ages. For example, on day 5, hemolymph JHE activity was approximately sixfold higher in LW versus SW *G. firmus*. Paired t-tests of hemolymph JHE activity documented that LW activities were significantly higher than SW activities on each of these three days (day 3, t = 13.4, P < 0.006; day 5, t = 13.1, P < 0.01; day 8, t = 5.7, P < 0.03). In contrast, activities were very similar for LW and SW females on the two days of adulthood. Although LW females had significantly higher hemolymph JHE activity than did SW females on day 3 of adulthood (t = 5.8, P < 0.03), the magnitude of the activity differences between morphs (1.2 fold) was substantially less than the sixfold difference between juvenile morphs. JHE activities did not differ between adult morphs on day 7 (t = 0.78, P > 0.5).

TABLE 3. Percentage of whole-cricket juvenile hormone esterase activity found in the hemolymph compartment of long-wing- (LW) and short-wing- (SW) selected *Gryllus firmus*. Sample sizes ranged from eight to 21 individuals, except for the following: block 1 SW day-5 (n = 6); block 2 SW day-8 (n = 5) and day-3 (n = 4). See Methods for additional experimental details.

Age	Morph	Block 1	Block 2	Block 3
Day-5 juvenile	LW	59.9 ± 3.2	61.1 ± 5.6	43.9 ± 3.3
	SW	13.8 ± 4.4	29.0 ± 3.4	18.2 ± 3.6
Day-8 juvenile	LW	28.6 ± 3.2	29.8 ± 2.7	29.8 ± 3.4
	SW	13.0 ± 3.8	10.3 ± 5.6	13.6 ± 3.3
Day-3 adult	LW	22.4 ± 2.7	25.2 ± 3.2	17.7 ± 1.8
	SW	16.7 ± 4.1	13.4 ± 6.4	10.4 ± 2.1
Day-7 adult	LW	8.5 ± 0.9	16.6 ± 3.3	12.2 ± 1.8
	SW	8.5 ± 1.4	10.6 ± 4.4	8.1 ± 2.4

Whole-cricket JHE activities were much more variable among LW females of L-lines compared with SW females from S-lines than were hemolymph JHE activities (Fig. 2). Like hemolymph JHE activities, whole-cricket JHE activities were the highest on day 5 of the last stage in LW individuals. In contrast to hemolymph JHE activities, whole-cricket JHE activities rose in all lines in day-7 adults compared with day-3 adults. Paired t-tests documented that JHE activities were higher in LW compared with SW morphs on day 5 (t = 20.0; P <0.01), but did not differ on day 8 (t = 2.3, P > 0.1) of the last stage. Even though JHE activities did not differ significantly on day 8, LW females in two of the three blocks had considerably higher enzyme activity compared with SW females. The lack of significance between mean JHE activities of LW versus SW females of this age was due to the low JHE activity of block-3 females, which considerably increased the variance of mean JHE activity among the L-lines. Least-squared mean JHE activities derived from pooled data for days 5 and 8 were significantly higher for LW (23.2 ± 1.9) compared with SW females (14.5 ± 0.4; t = 5.45, P < 0.05). Thus, whole-cricket JHE activity is higher in LW compared with SW selected females when all data obtained on last-stage crickets are considered. In contrast, whole-cricket JHE activities did not differ between LW and SW morphs on either day 3 (t = 2.7, P > 0.1) or day 7 of adulthood (t = 1.2, P > 0.1).

Genetic Differences in Tissue Distribution between Long-Winged and Short-Winged Morphs

A considerably higher percentage of whole-cricket JHE activity was found in the hemolymph of LW compared with SW morphs on both day 5 (t = 5.74, P < 0.05) and day 8 (t = 14.1, P < 0.01) of the last stage (Table 3). For example, on day 5 about 60% of JHE activity was found in the hemolymph of LW females, while only 18% of JHE activity was found in the hemolymph of SW females. In contrast, JHE activity was elevated to a much lesser degree in the blood of LW vs. SW adult females. For neither day-3 females (t = 3.08, P > 0.05)

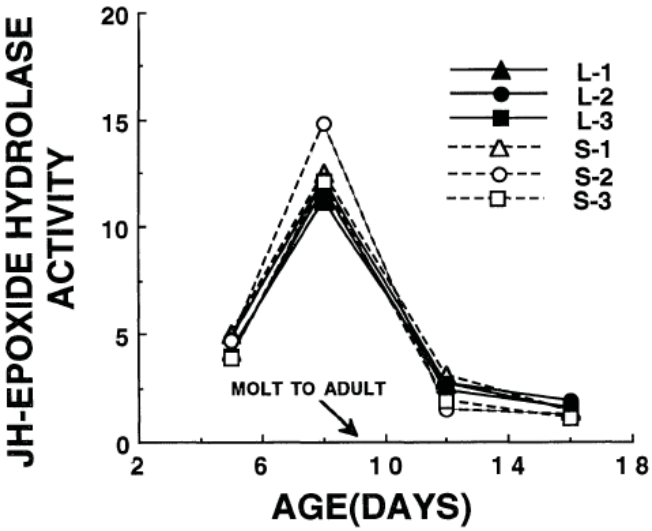

Figure 3. Mean whole-organism juvenile hormone eposide cydrolase activity in long-winged (LW) females from the LW-selected (L) lines and short-winged (SW) females from the SW-selected (S) lines during the last juvenile stage and first week of adulthood of *Gryllus firmus.* Symbols and ages are the same as those in Figure 1. Activities are in pmol JH diol/min mg wet weight. Sample sizes are the same as those in Figure 2. For clarity, error bars are not shown; average SEM as percent of the mean was 8.8 ± 1.0%.

nor day-7 females (t = 1.9, P > 0.1) were the differences in tissue distribution between LW and SW morphs significant.

Juvenile Hormone Epoxide Hydrolase Activity

JH epoxide hydrolase activity exhibited virtually identical developmental profiles in each of the six selected lines (Fig. 3). Profiles differed from JHE developmental profiles. The peak of JH epoxide hydrolase activity occurred near the end of the last stage, whereas the JHE activity peak occurred at mid-stage. Paired t-tests indicated no significant difference in JH epoxide hydrolase activity between LW and SW females from a particular block on any day (day-5 juveniles, t = 0.66, P > 0.1; day-8 juveniles, t = 2.1, P > 0.1; day-3 adults, t = 0.93, P > 0.1; day-7 adults, t = 2.6, P > 0.5). Therefore, no genetic differences in JH epoxide hydrolase activity were documented between LW and SW morphs from the selected lines.

In Vivo Juvenile Hormone Degradation

Mean percent JH-III degraded *in vivo* in juvenile and adult LW and SW females from the three blocks are presented in Figure 4. In each block, *in vivo* JH-III degradation was higher in LW compared with SW females both in the last stage and in adults. Paired t-tests documented significant genetic differentiation for JH degradation between wing morphs on day 5 of the last stage (t = 7.1, P < 0.025), but not on day 7 of adulthood (t = 2.84, P > 0.1). JH degradation rates were similar between LW adults and LW juveniles, but were higher in SW adults compared with SW juveniles.

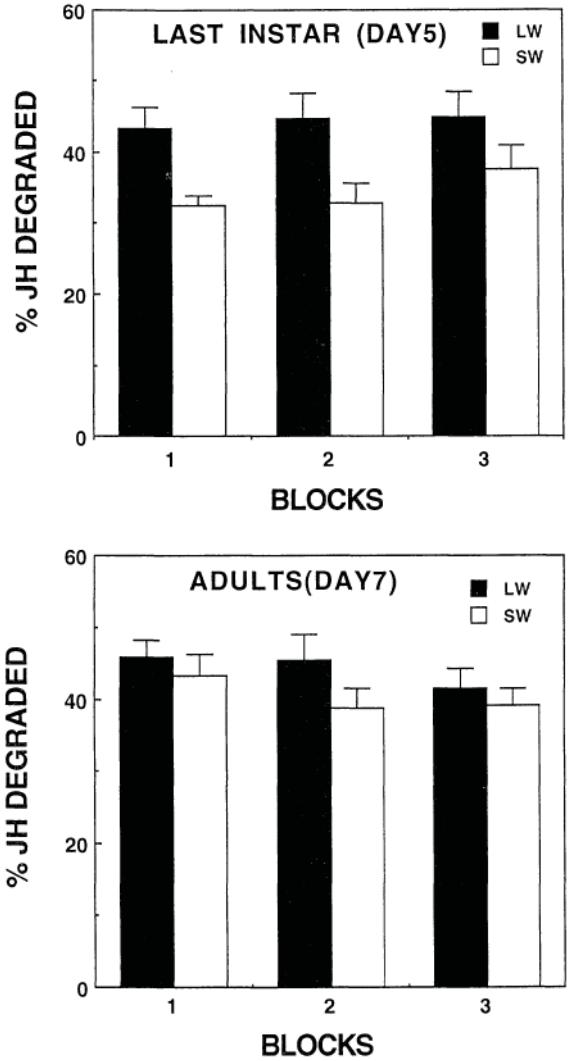

Figure 4. Mean (± SEM) percentage of radiolabeled juvenile hormone degraded *in vivo* 50 min after injection into long-winged (LW) females from the LW-selected (L) lines or short-winged (SW) females from the SW-selected (S) lines of *Gryllus firmus.* Each mean is based on assays of six to 12 individual crickets (median = 10).

DISCUSSION

JHE in *Gryllus* has become the most intensively studied model in evolutionary endocrinology. This is the case both with respect to the quantitative genetics of endocrine variation and covariation per se (*e.g.,* Zera and Zhang 1995; Gu and Zera 1996; Zera *et al.* 1996, 1998) and the functional relationship between genetically variable endocrine and whole-organism (morphological, life-history) traits (*e.g.*, Zera and Tiebel 1989; Zera *et al.* 1989; Fairbairn and Yadlowski 1997; Roff *et al.* 1997; Zera and Denno 1997). The present study of morph-specific variation for JHE and related endocrine features in *G. firmus* considerably extends results of previous work on this topic. Our earlier studies of endocrine variation in the congener *G. rubens* were restricted to a single pair of LW- and SW-selected lines, except for the case of hemolymph JHE activity,

where enzyme activity was also measured in F_1 and F_2 crosses between those lines (reviewed in Zera and Denno 1997; Zera 1999; see Introduction). More recent work in *G. firmus* has characterized pairs of LW-selected, SW-selected, and control lines, as well as half-sib families (Fairbairn and Yadlowski 1997; Roff *et al.* 1997). However, these studies were restricted to a single endocrine trait (hemolymph JHE activity) in juveniles. The present study investigated a more diverse set of endocrine traits in both adult and juvenile wing morphs of *G. firmus* that were derived from three pairs of LW- and SW-selected lines. Three traits, whole-organism JHE activity, JHE tissue distribution, and JH-epoxide hydrolase activity, are the first to be studied in any wing-polymorphic insect. This study also provides the first demonstration that genetically differentiated wing morphs that vary in JHE activity also differ in JH metabolism. This is a key link in the chain of causality from variation in JHE activity to variation in JH-mediated wing morph expression.

Endocrine Genetics of Wing Polymorphism in Gryllus firmus

Juvenile Stage.—The substantially elevated hemolymph JHE activity in LW versus SW *G. firmus* on each of three days of the last juvenile stage (Figs. 1,5) is similar to results found for *G. rubens* (Zera and Tiebel 1989), independently selected lines and half-sib families of *G. firmus* (Fairbairn and Yadlowski 1997; Roff *et al.* 1997), and, to a lesser degree, selected lines of *Modicogryllus confirmatus* (Zera and Tanaka 1996). These data suggest that morph-associated genetic variation for JHE activity may be common in wing-polymorphic crickets, at least those of the related genera *Gryllus* and *Modicogryllus.* These data clearly demonstrate that JHE either directly regulates or is strongly associated with loci that regulate the determination or differentiation of traits that vary between wing morphs of *G. firmus* (*e.g.*, length of wings, development of flight muscles).

A convincing argument that variation in JHE activity directly causes variation in wing morph development must include a demonstration that altered JHE activity, measured *in vitro*, results in altered JH catabolism, measured *in vivo*. Only if variation in JHE activity changes the rate at which JH is degraded *in vivo* can JHE alter the JH titer and, thus, affect wing morph development. It is well known that even dramatic variation in enzyme activity (50% or greater) sometimes does not have any measurable physiological consequences (Kascer and Burns 1981; fig. 5 of Dykhuizen and Dean 1990). This results from the many compensatory mechanisms that can dampen the effect of variation in enzyme activity on higher-level physiological processes such as pathway flux (Kascer and Burns 1981). Therefore, it is inappropriate to simply assume that variation in JHE activity necessarily results in variation in JH metabolism, as has been done by Fairbairn and Yadlowski (1997) and Roff *et al.* (1997; for a critical review of these studies, see Zera 1999). Indeed, during the early stages (sixth generation) of direct selection on hemolymph JHE activity in the cricket *Gryllus assimilis,* most high-activity lines differed from low-activity lines by several fold in JHE activity without differing significantly in JH degradation *in vivo* (Zera and

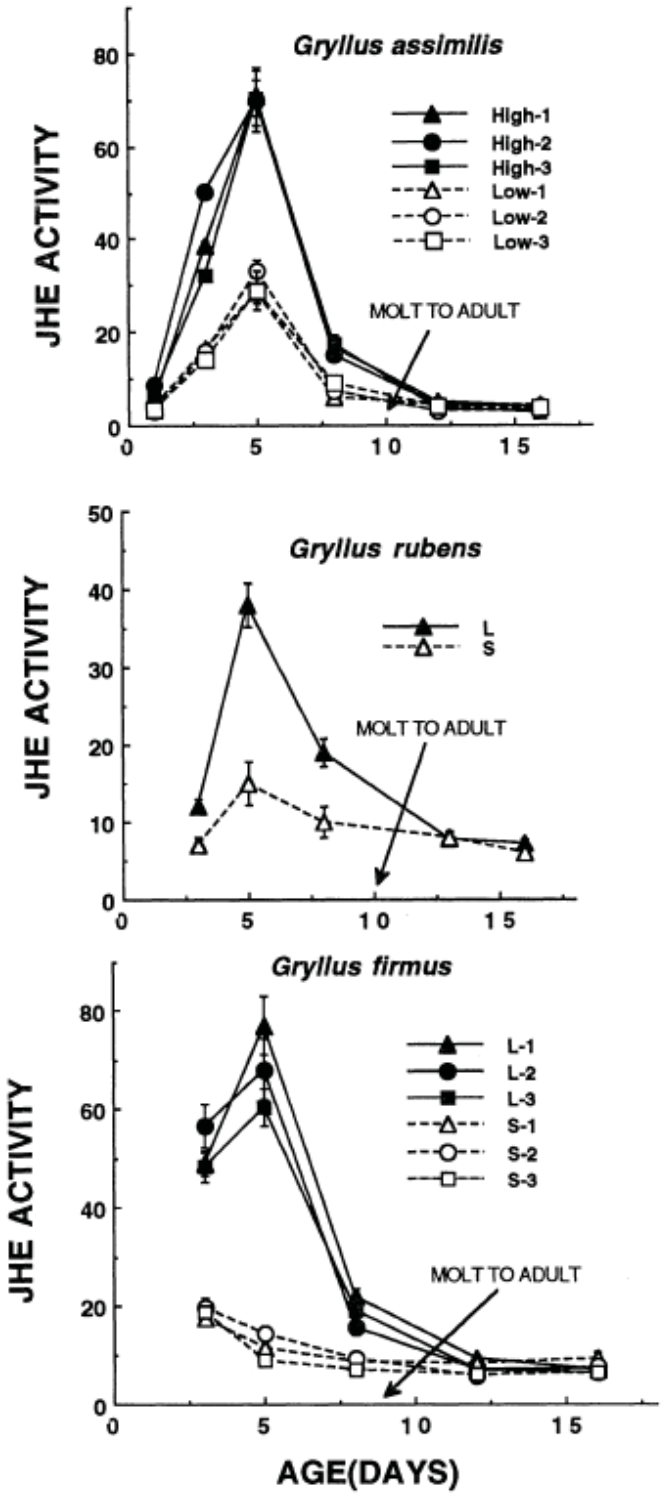

Figure 5. Hemolymph juvenile hormone esterase activities (nmol/min ml) in three species of *Gryllus* during the last stage and first week of adulthood. For *G. assimilis,* "High" and "Low" refer to lines directly selected for high or low hemolymph JHE activity. For *G. rubens* and *G. firmus,* "L" and "S" refer to long-wing and short-wing selected lines, respectively. Data for *G. assimilis* are from Zera and Zhang (1995), for *G. rubens,* data are from Zera and Tiebel (1989), and for *G. firmus,* data are from Figure 1. In each case, day 0 refers to the first day of the last juvenile stage.

Zhang 1995; Zera 1999). The demonstration that genetically differentiated wing morphs of *G. firmus* differ in JH metabolism (Fig 4; Results) provides the first direct evidence for this species that variation in *in vitro* JHE activity has important physiological consequences with respect to modulation of the JH titer *in vivo*. These data also represent the first demonstration of naturally occurring genetic variation for hormone metabolism in an insect and compliment previous data on phenotypic variation in JH degradation between wing morphs of the congener *G. rubens* (Zera and Holtmeier 1992).

Associations among whole-organism and hemolymph JHE activity, tissue distribution in JHE activity, whole-organism JH epoxide hydrolase activity, and wing morph in *G. firmus* provide new insights into the endocrine genetics of wing polymorphism. These data indicate that elevated hemolymph JHE activity in LW versus SW *G. firmus* activity results from two causes: increased whole-organism enzyme activity in LW individuals and increased percentage of whole-organism JHE activity found in the hemolymph compartment of that morph. Thus, polymorphic genetic factors that regulate the development of different wing morphs in *G. firmus* are either strongly associated with or are identical to genetic factors that regulate both the concentration of whole-organism JHE activity and its tissue distribution. At present we have no information on the identity of these loci or their gene products in *G. firmus,* although some data are available for *G. rubens* and *G. assimilis* (see below).

In contrast to the dramatic variation between morphs in hemolymph and whole-organism JHE activity, we observed no difference between morphs in the activity of JH epoxide hydrolase, the other major JH metabolizing enzyme (Fig. 3). Thus, only one of the two major enzymatic regulators of whole-organism JH degradation exhibits polymorphic variation in activity that is associated with genetically specified wing morph. The significance of this result is currently unknown. Because whole-organism JHE and JH epoxide hydrolase activity and tissue distribution of JHE have not been quantified in other wing-polymorphic insects, the extent to which these morph-specific associations in *G. firmus* also occur in other wing-polymorphic species is currently unknown. Both whole-organism JHE and JH epoxide hydrolase activities have been studied in several nonpolymorphic insects (Zera and Zhang 1995; de Kort and Granger 1996; Zera *et al.* 1996; see below).

Adult Stage and Adult-Juvenile Interaction.—Two central issues in the evolution and regulation of wing polymorphism involve the adult stage. The first concerns the endocrine mechanisms responsible for variation in ovarian growth between adult wing morphs (Table 2). The second concerns the mechanisms that coordinate the development of a specific wing morph during the juvenile stage with the expression of functionally appropriate features of that morph during the adult stage (*e.g.*, earlier onset of ovarian growth in flightless, SW females). The present study provides no indication that variation in JHE activity is involved in either of these processes. In contrast to the dramatically higher hemolymph and whole-body JHE activity in LW-selected versus SW-selected *G. firmus* during the last juvenile stage, adult wing morphs that differed in ovarian growth (Table 2) showed no consistent differences or only minor differences in hemolymph JHE activity, whole-body JHE activity, and JH-epoxide hydrolase activity (Figs. 1–3). The existence of substantial differences in hemolymph JHE activities between juvenile but not between adult-wing morphs in *G. firmus* is not unexpected. Hemolymph JHE activity exhibits no significant additive genetic correlation between adults and juveniles in the congener, *G. assimilis* (Zera *et al.* 1998; see below). As predicted by the JH/wing polymorphism hypothesis (Zera and Denno 1997), we have recently documented that the JH titer is significantly higher in SW compared with LW *G. firmus* adults and that the JH titer is positively correlated with ovarian growth (G. Cisper and A. J. Zera, unpubl. data). Thus, some mechanism other than the differential degradation of JH appears to be responsible for producing variation in JH titers and ovarian growth between adult wing morphs.

Our conclusions of the preceding paragraph do not concur with those of Fairbairn and Yadlowski (1997) and Roff *et al.* (1997), who argued that variation in JHE activity in juvenile wing morphs causes variation in ovarian growth (and other adult traits) between adult wing morphs of *G. firmus.* Those authors did not measure JHE activity in adult wing morphs of *G. firmus.* Nor did they provide any empirical data or an explicit model as to how a particular level of JHE activity in *juveniles* might affect the onset of ovarian growth in *adult* morphs of *G. firmus.* Their argument is solely based on the observation that JHE activity in juveniles exhibits a strong genetic correlation with ovarian mass in adult morphs. Because traits can be strongly correlated without being causally related, an argument of causality that is based solely on the existence of a correlation is inherently weak. In support of their argument, these authors further stated that variation in JHE can affect adult reproduction "indirectly by shifting subsequent developmental pathways" (Roff *et al.* 1997, p. 1912) and that "Such indirect effects of hormones in the juvenile stages on adult phenotypes are well documented in insects (Hardie and Lees 1985; Pener 1985; Nijhout 1994)" (as cited in Roff *et al.* 1997, p. 1912; Fairbairn and Yadlowski 1997). However, the adult traits discussed in these cited reviews are those such as the length of the wings that develop during the *juvenile stage.* It is easy to see how JHE during the juvenile stage can affect an adult trait that develops during the juvenile stage (wings). It is less clear how JHE in juveniles might affect the growth of the ovaries, which occurs almost exclusively in adults. However, it is easy to envision the null model, that JHE activity in juveniles is correlated with but has no causal influence on ovarian growth, because both JHE activity in juveniles and the various factors that regulate ovarian growth in adults (*e.g.*, regulators of the adult JH titer) are themselves regulated by neurohormones (Roe and Venkatesh 1990; Nijhout 1994; Wyatt and Davey 1996). The endocrine-genetic mechanisms that coordinate wing morph development and wing morph reproduction remain one of the most intriguing but least understood aspects of wing polymorphism.

Comparative Endocrine Genetics of Juvenile Hormone Esterase

Endocrine differences between LW-and SW-selected morphs of *G. firmus* are remarkably similar to differences be-

Table 4. Comparison of endocrine variation between lines of *Gryllus assimilis* selected for high or low juvenile hormone esterase (JHE) activity and long-wing- (LW) or short-wing- (SW) selected *G. firmus*. Values represent means (±SEM) of three lines selected for high or low JHE activity (*G. assimilis*) or means of LW (or SW) individuals in three lines selected for the LW (or SW) morph in *G. firmus*. Data for *G. assimilis* are from Zera *et al.* (1996, 1998; seventh to 10th generations of selection). Data for *G. firmus* are from Figures 1–4 and Table 3. Unless otherwise stated, traits were measured on day 3 (*G. assimilis*) or day 5 (*G. firmus*) of the last juvenile stage. See Figures 1–4 and Table 3 for units of various measurements. JHEH activity, whole-organism juvenile hormone epoxide hydrolase activity.

	G. assimilis		*G. firmus*	
Trait	High	Low	LW	SW
Hemolymph JHE activity	57.1 ± 2.6	8.4 ± 0.63	68.6 ± 4.6	11.5 ± 1.5
Whole-organism JHE activity	10.7 ± 1.5	4.4 ± 0.55	28.4 ± 1.7	15.6 ± 2.0
Percent JHE activity in hemolymph	71.5 ± 2.0	27.8 ± 1.5	55.0 ± 5.3	20.3 ± 4.3
JHEH activity	11.2 ± 0.96	9.6 ± 0.33	4.33 ± 0.23	4.5 ± 0.35
JH degradation	60.7 ± 4.8	43.2 ± 7.1	44.4 ± 0.52	34.3 ± 1.64
Adult hemolymph JHE activity (day 2/3)	4.8 ± 0.8	2.6 ± 0.2	7.7 ± 0.65	6.8 ± 0.80

tween lines of the long-wing, monomorphic cricket *G. assimilis* that were directly selected for elevated or decreased hemolymph JHE activity (summarized in Table 4). For example, in LW-selected versus SW-selected *G. firmus* and in high versus low JHE activity lines of *G. assimilis* (seventh to 10th generations of selection), hemolymph JHE activity was elevated 6–8-fold, whole-cricket JHE activity was elevated 2.4–1.8-fold, and percentage JHE in the hemolymph compartment was elevated 2.6-fold. Furthermore, both whole-cricket JH epoxide hydrolase activities in juveniles and adults and JHE activities in adults did not differ substantially between selected lines of *G. assimilis* as was the case for *G. firmus* (Figs. 1–5; Table 4). These data on the comparative endocrine genetics of JHE and functionally related traits in these congeners provide important information on the both the evolution of endocrine regulation and the evolution of wing polymorphism.

In *G. assimilis* no genetic correlation was observed between JHE and JH epoxide hydrolase activities (Zera and Zhang 1995; Table 4). A similar lack of genetic correlation between these two traits in *G. firmus* would explain why both hemolymph JHE activity and JH epoxide hydrolase activity are not genetically associated with wing morph. The presence or absence of a genetic correlation between JHE and JH epoxide hydrolase in *G. firmus* cannot be directly determined from data currently available because both of these traits are indirect responses to selection on wing morph. In selection studies, genetic correlations can only be determined between traits, if one of the traits is directly selected (Falconer 1989).

In *G. assimilis* and *G. rubens,* the JHE enzymes from genetic stocks differing in JHE activity have been extensively characterized. Thus far no kinetic or stability differences have been found between JHEs from these lines, indicating that JHE activity differences are almost certainly not due to morph- or line-specific allozymes (Zera *et al.* 1992; Zera and Zeisset 1996). Rather, results to date suggest that JHE activity differences are due to variation in "regulatory loci" that control the synthesis, degradation, or activity of JHE in a morph- or line-specific manner. The same is likely true for JHE activity differences between LW- and SW-selected morphs of *G. firmus* (Figs. 1,2). We are currently in the process of isolating JHE cDNAs from *G. assimilis*. If successful. these cDNAs will be useful as probes to investigate transcriptional regulation of JHE activity in LW- and SW-selected *G. firmus.*

In *G. assimilis,* strong genetic correlations exist for hemolymph JHE activities between different days of the last stage or between different days of adulthood. However, no measurable genetic correlation exists between activities in adults and juveniles (Zera and Zhang 1995; Gu and Zera 1996; Zera *et al.* 1998). In *G. assimilis* this gives rise to JHE developmental profiles in which hemolymph JHE activities differ dramatically between JHE selected lines on all days of the last stage but are virtually identical on all days of adulthood (Fig. 5). The similar developmental profiles for hemolymph JHE activity in *G. assimilis, G. firmus,* and *G. rubens* (Fig. 5) suggest that a similar pattern of genetic correlations may exist in these three species for loci affecting hemolymph JHE activities on different days of adult or juvenile development. This, in turn, suggests that these correlations have been conserved since the evolutionary divergence of these three species. The extent to which genetic correlations are stable over evolutionary time remains an important but unresolved issue in evolutionary genetics (Turelli 1988; Wilkinson *et al.* 1990; Price and Langen 1992). Endocrine covariation in species of *Gryllus* may be an excellent model to address this issue, especially because the functional basis of these correlations are amenable to study.

The Functional Relationship between Juvenile Hormone Esterase Activity and Wing Morph Development in Gryllus firmus*: Caveats and Conclusions*

The present study has provided several important pieces of evidence consistent with the hypothesis that genetically based wing morphs are produced by genetic variation in JHE activity during the last juvenile stage. However, information obtained to date on *G. firmus* and congeners also is consistent with the null hypothesis that JHE activity is correlated with but not causally involved in wing morph development. Many enzymes are regulated by similar factors (*e.g.*, cofactors, hormones or transcription factors; Harshman and James 1998). Thus, it is possible that the association between JHE activity and wing morph might result from a nonfunctional response to some factor that controls the true regulator(s) of wing morph development. As long as variation in JHE activity does not reduce fitness, such an incidental association would not be selected against. A similar argument has been made that interspecific variation in the tissue expression of glucose dehydrogenase in *Drosophila* species is an "incidental rather than adaptive" response to regulators of the sex determination hi-

erarchy (Cavener *et al.* 1987; cited in Harshman and James 1998). Thus, in contrast to statements made by Fairbairn and Yadlowski (1997), the mere correlation between JHE activity and wing morph does not necessarily *demonstrate* its importance in the regulation of wing dimorphism. Current knowledge of the mechanisms that specify genetic or environmental variation in wing polymorphism or functionally related types of polymorphism, such as phase polymorphism in locusts or caste polymorphism in social insects, is very rudimentary. This increases the possibility that nonfunctional correlations will be mistaken for casual relationships and dictates caution in postulating physiological explanations for morph expression (Zera 1999).

Several key pieces of information in addition to those provided in the present study are required before a causal relationship between JHE activity and wing morph development can be considered to be strongly supported in *G. firmus*. For example, no information has been published showing that JH itself affects wing morph development in this species. Clearly, JHE can only influence wing morph development via its effect on the JH titer if JH itself regulates wing morph development. Such a role for JH must be established by documenting that: (1) the *in vivo* JH titer differs between nascent wing morphs: and (2) experimental manipulation of the JH titer alters morph development in a predictable manner. We are currently undertaking such studies. In addition, the importance of variation in JHE and JH titers in regulating wing morph development can only be assessed in the context of information on the importance of variation in the other hormones (*e.g.*, ecdysteroids, neurohormones; Zera *et al.* 1989; Zera 1999). Clearly, the identification of endocrine correlates of wing polymorphism in *G. firmus* and *G. rubens* during the past decade represents an important advance in understanding the potential physiological-genetic basis of ecologically important phenotypic variation. However, thoroughly documenting the actual importance of the various candidate hormonal regulators will be a major challenge for future research.

ACKNOWLEDGMENTS

Research reported here was supported by grant IBN-9507388 from the National Science Foundation. YH was supported by a Visiting Scholar Fellowship from the Peoples Republic of China and a supplement to the National Science Foundation grant listed above

LITERATURE CITED

Alexander, R. D. 1968. Life cycle origins, specialization and related phenomena in crickets. Q. Rev. Biol. 43:1–41.

Alexander, R. D., and T. J. Walker. 1962. Two introduced field crickets new to eastern United States (Orthoptera: Gryllidae). Ann. Entomol. Soc. Am. 55:90–94.

Caveser, D. J., M. Martha, C. Schonbaum, and D. Hollar. 1987. Cell autonomous and hormonal control of sex-limited gene expression in *Drosophila.* Pp 453–462 *in* J. H. Law, ed. Molecular entomology. Liss, New York.

De Kort, C. A. D., and N. A. Granger. 1996. Regulation of JH titers: the relevance of degradative enzymes and binding proteins. Arch. Insect Biochem. Physiol. 33:1–26.

Dingle, H. 1996. Migration. The biology of life on the move. Oxford Univ. Press, New York.

Dykhuizen, D. E., and A. M. Dean. 1990. Enzyme activity and fitness: evolution in solution. Trends Ecol. Evol. 5:257–262.

Fairbairn, D. J., and D. E. Yadlowski. 1997. Coevolution of traits determining migratory tendency: correlated response of a critical enzyme, juvenile hormone esterase, to selection on wing morphology. J. Evol. Biol. 10:495–513.

Falconer, D. S. 1989. Introduction to quantitative genetics. 3rd ed. Longman Scientific and Technical, New York.

Gould, S. J. 1977. Ontogeny and phylogeny. Belknap Harvard Press, Cambridge, MA.

Gu, X., and A. J. Zera. 1996. Quantitative genetics of juvenile hormone esterase, juvenile hormone binding, and general esterase activity in the cricket, *Gryllus assimilis.* Heredity 76:136–142.

Hammock, 1985. Regulation of the juvenile hormone titer: B. D. degradation. Pp. 43 1–472 *in* G. A. Kerkut and L. I. Gilbert, eds. Comprehensive insect physiology, biochemistry and pharmacology. Vol. 7. Pergamon Press, New York.

Hammock, B. D., and T. C. Sparks. 1977. A rapid assay for juvenile hormone esterase activity. Analyt. Biochem. 82:573–579.

Hardie, J., and A. D. Lees. 1985. Endocrine control of polymorphism and polyphenism. Pp. 441–490 *in* G. A. Kerkut and L. I. Gilbert, eds. Comprehensive insect physiology, biochemistry and pharmacology. Vol. 8. Pergamon Press, Oxford.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annu. Rev. Ecol. Syst. 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, songs and habitat associations. J. Evol. Biol. 8:209–232.

Harshman, L. G., and A. A. James. 1998. Differential gene expression in insects: transcriptional control. Annu. Rev. Entomol. 43:671–700.

Kascer, H., and D J. A. Burns, 1981. The molecular basis of dominance. Genetics 97:639–666.

Nijhout, H. F. 1994. Insect hormones. Princeton Univ. Press, Princeton, NJ.

Price, T., and T. Langen. 1992. Evolution of correlated characters. Trends Ecol. Evol. 7:307–310.

Roe, R. M., and K. Venkatesh. 1990. Metabolism of juvenile hormones: degradation and titer regulation. Pp. 126–179 *in* A. P. Gupta, ed. Morphogenetic hormones of arthropods, Rutgers Univ. Press, New Brunswick, NJ.

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

Roff, D., G. Stirling, and D. J. Fairbairn. 1997. The evolution of threshold traits: a quantitative genetic analysis of the physiological and life-history correlates of wing dimorphism in the sand cricket. Evolution 51:1910–1919.

Rose, M. R., T. J. Nusbaum, AND A. K. Chippendale. 1996. Laboratory evolution: the experimental wonderland and the cheshire cat syndrome. Pp. 221–244 *in* M. R. Rose and G. V. Laudel; eds. Adaptation. Academic Press, San Diego, CA.

Share, M. R., and R. M. Roe. 1988. A partition assay for the simulta-

neous determination of insect juvenile hormone esterase and epoxide hydrolase activity. Analyt. Biochem. 169:81–88.

Sokal, R. R., and F. J. Rohlf. 1989. Biometry. 2d ed. Freeman, San Francisco, CA.

Southwood, T. R. E. 1961. A hormonal theory of the mechanism of wing polymorphism in Heteroptera. Proc. R. Ent. Soc. Lond. 36:63–66.

Turelli, M. 1988. Phenotypic evolution, constant covariances, and the maintenance of additive genetic variance. Evolution 42: 1342–1347.

Veazy, J. N., C. A. R. Kay, T. J. Walker, and W. H. Witcomb. 1976. Seasonal abundance, sex ratio, and macroptery of field crickets in northern Florida. Ann. Entomol. Soc. Am. 69:374–380.

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

Wilkinson, G. S., K. Fowler, and L. Partridge. 1990. Resistance of genetic correlation structure to directional selection in *Drosophila melanogaster.* Evolution 44: 1990–2003.

Wyatt, G. R., and K. G. Davey. 1996. Cellular and molecular actions of juvenile hormone. 11. Roles of juvenile hormone in adult insects. Adv. Insect Physiol. 26:1–155.

Zera, A. J. 1999. The endocrine genetics of wing polymorphism: Critique of recent studies and state of the art. Evolution 53:972–976.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annu. Rev. Entomol. 42:207–231.

Zera, A. J., and C. L. Holtmeier. 1992. *In vivo* and *in vitro* degradation of juvenile hormone-III in presumptive long-winged and short-winged *Gryllus rubens.* J. Insect Physiol. 38:61–74.

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.

Zera, A. J., and S. Tanaka. 1996. The role of juvenile hormone and juvenile hormone esterase in wing morph determination in *Modicogryllus confirmatus.* J. Insect Physiol. 42:909–915.

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

———. 1989. Differences in juvenile hormone esterase activity between presumptive macropterous and brachypterous *Gryllus rubens:* implications for the hormonal control of wing polymorphism. J. Insect Physiol. 35:7–17.

Zera, A. J., and M. Zeisset. 1996. Biochemical characterization of juvenile hormone esterases from lines selected for high or low enzyme activity in *Gryllus assimilis.* Biochem. Genet. 34: 421–435.

Zera, A. J., and C. Zhang. 1995. Direct and correlated responses to selection on hemolymph juvenile hormone esterase activity in *Gryllus assimilis.* Genetics 141: 1125–1134.

Zera, A. J., C. Strambi, K. C. Tiebel, A. Strambi, and M. A. Rankin. 1989. Juvenile hormone and ecdysteroid titers during critical periods of wing morph determination in *Gryllus rubens.* J. Insect Physiol. 35:501–511.

Zera, A. J., X. Gu, and M. Zeisset. 1992. Characterization of juvenile hormone esterase from genetically determined wing morphs of the cricket, *Gryllus rubens.* Insect Biochem. Mol. Biol. 22:829–839.

Zera, A. J., C. Borcher, and S. B. Gaines. 1993. Juvenile hormone degradation in adult wing morphs of the cricket, *Gryllus rubens.* J. Insect Physiol. 39:845–856.

Zera, A. J., J. Sall, and R. Schwartz. 1996. Artificial selection on JHE activity in *Gryllus assimilis:* nature of activity differences between lines and effect on JH binding and metabolism. Arch. Insect Biochem. Physiol. 32:421–428.

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. Physiol. Zool. 70:519–529.

Zera, A. J., T. Sanger, and G. L. Cisper. 1998. Direct and correlated responses to selection on JHE activity in adult and juvenile *Gryllus assimilis:* implications for stage-specific evolution of insect endocrine traits. Heredity 80:300–309.

Corresponding editor for the print journal edition: C. Boggs