Evolutionary Endocrinology of Juvenile Hormone Esterase in *Gryllus assimilis*: Direct and Correlated Responses to Selection

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ABSTRACT

Hemolymph juvenile hormone esterase (JHE) activity on the third day of the last stadium in the cricket, *Gryllus assimilis*, exhibited a significant response to selection in each of six replicate lines. Mean realized heritability was 0.26 ± 0.04. The response was due to changes in whole-organism enzyme activity as well as to changes in the proportion of enzyme allocated to the hemolymph compartment. *In vivo* juvenile hormone metabolism differed between some lines selected for high *vs.* low enzyme activity. Only minimal differences were observed between lines with respect to hemolymph protein concentration or whole-cricket activity of juvenile hormone epoxide hydrolase, the other major JH-degrading enzyme. Dramatic correlated responses to selection, equal in magnitude to the direct response, were observed for JHE activity on each of three other days of the last juvenile stadium. In contrast, no correlated responses in JHE activity were observed in adults. This indicates that JHE activities throughout the last stadium will evolve as a highly correlated unit independent of adult activities and the evolution of endocrine mechanisms regulating juvenile development can be decoupled from those controlling adult reproduction. This study represents the first quantitative-genetic analysis of naturally occurring endocrine variation in an insect species.

DURING the past three decades a tremendous amount of chemical, biochemical and physiological information has been obtained on insect endocrine characteristics (DOWNER and LAUFER 1983; KERKUT and GILBERT 1985; GUPTA 1990). However, genetic aspects of insect endocrinology, especially population-genetic aspects, have been much less studied. Except for a few rare cases (see below), there are currently no published data on the amount, characteristics or degree of interaction among naturally occurring, genetically based endocrine variations. Because of this paucity of population-genetic information on hormone titers, hormone receptors or activities of enzymes involved in hormone biosynthesis or degradation, our understanding of the microevolutionary processes that modify or constrain the insect endocrine system is limited.

Most physiological traits in either natural or unselected, outbred laboratory populations appear to have a polygenic mode of inheritance. These range from whole-organism traits such as locomotor activity and desiccation tolerance to enzyme activities and concentrations of energy reserves (LAURIE-AHLBERG *et al.* 1980, 1982; ARNOLD 1987; CLARK and KEITH 1988; GARLAND 1988; BENNETT and HUEY 1990; GARLAND and CARTER 1994). A polygenic mode of inheritance also appears to be common for unselected endocrine variation in laboratory strains of domestic stocks of vertebrates (SHIRE 1979). Quantitative-genetic methodologies are the most appropriate for analyzing this type of genetic variation. In the present study, we used a conventional artificial selection experiment to investigate genetic variation and covariation in a model insect endocrine character, juvenile hormone esterase (JHE) activity, in the cricket *Gryllus assimilis*.

JHE is a hydrolytic enzyme that degrades the key developmental and reproductive hormone, juvenile hormone (JH) (HAMMOCK 1985; ROE and VENKATESH 1990). The activity of this enzyme increases dramatically during the last stadium when a reduction in the JH titer to very low levels is required for metamorphosis to proceed. Numerous biochemical and physiological studies have implicated a role for JHE in the regulation of the JH titer (HAMMOCK 1985; ZERA and TIEBEL 1989; ROE and VENKATESH 1990; ZERA and HOLTMEIER 1992).

We focused on JHE activity in *G. assimilis* for a variety of reasons. First, as described above, JHE is functionally important by virtue of its role in modulating the JH titer. Second, in contrast to all other insect endocrine traits, a large data base is available on naturally occurring genetic variation in JHE activity in the closely related cricket, *G. rubens*. *G. rubens* is dimorphic for dispersal capability, and JHE has been extensively studied in the context of the regulation of the JH titer and morph determination (ZERA and TIEBEL 1989; ZERA *et al.* 1993 and references therein). This background information allows interpretation of the JHE selection experiments in *G assimilis* to a degree not possible for other less-studied endocrine characters. Finally, JHE

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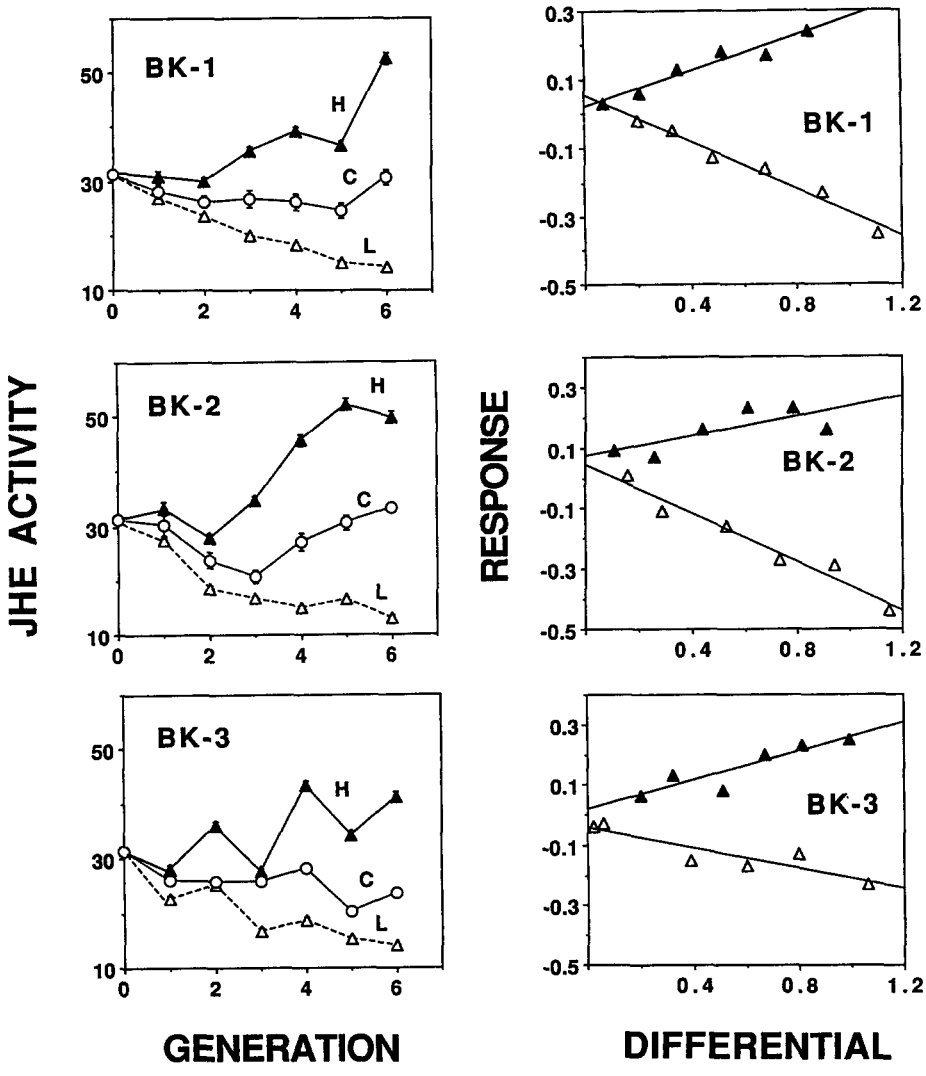

FIGURE 1.—Results of selection on day 3 hemolymph JHE activity in *G. assimilis*. Left panels: Line means during each generation of selection in the three blocks (BK, block; H, L, and C, high selected, low selected and control lines, respectively). Bars represent standard errors, which in many cases are smaller than the symbols. Enzyme activity = nmol JH-acid/min/ml hemolymph adjusted for stadium duration (see MATERIALS AND METHODS). Right panels: Response to selection *vs.* cumulative selection differential (log-transformed activities). Symbols are the same as in the left panels. Lines represent results of linear regression analyses. See Table 1 for heritabilities estimated from these analyses and MATERIALS AND METHODS for additional details.

has some practical advantages in quantitative-genetic studies of insect endocrine characters. The activity of the enzyme can be determined in a few microliters of hemolymph, thus allowing individuals whose enzyme phenotype has been determined to be bred in selection experiments. A relatively rapid radiochemical assay for JHE activity (HAMMOCK and SPARKS 1977) is available thus permitting analysis of the large number of samples required in a quantitative-genetic study. Finally, assays of many other endocrine factors that affect the JH titer such as the concentration/activity of JH binders, the activity of other JH-degrading enzymes such as JH-epoxide hydrolase and JH biosynthetic rate are available (PRATT and TOBE 1974; KOEPPE and KOVALICK 1986; SHARE and ROE 1988). This allows the study of genetic correlations among functionally related characters that influence the JH titer.

In the present study, JHE activity was selected in an outbred laboratory population of *G. assimilis* that had been recently initiated from field collected individuals. The study had three main goals. The first was to determine the response to selection and hence the degree to which a typical endocrine factor has the capacity to be altered by natural selection. Second, we measured correlated responses to selection on JHE activity at several juvenile and adult developmental stages. This was done to determine the degree to which endocrine traits expressed at different points in development are capable of evolving independently of each other. Finally we measured whole-cricket JH degradation *in vitro* and *in vivo* to assess the consequences of altering JHE activity on whole-organism hormone metabolism.

MATERIALS AND METHODS

Background and standard rearing conditions: *G. assimilis* 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). Crickets used in the present study were derived from 21 impregnated females collected at Homestead, FL, during the summer/fall 1992. Crickets were reared in the laboratory for 4–7 months (two to three generations) before the start of the selection experiment. During this time, the total population of breeding adults was kept between 250 and 300 individuals divided equally into 6–8 10-gallon aquaria.

Crickets were reared under standard conditions [*e.g.*, fed the dry diet described in ZERA and RANKIN (1989) and raised under a 16-hr light:8-hr dark photoperiod at 28°].

Selection experiment: The selection experiment consisted of three replicate blocks each of which contained one line selected for high enzyme activity, one line selected for low enzyme activity and a control line. The character selected was hemolymph juvenile hormone esterase (JHE) activity on day 3 of the last stadium. This age was chosen based on a pilot study which demonstrated significant heritability (half-sib analysis) of hemolymph JHE activity at this age ($h^2 = 0.57 \pm 0.27$) in a long-term laboratory population of *G. assimilis* (GU and ZERA 1996).

Lines were set up as follows. In the spring of 1993, hemolymph JHE activity was measured in ~729 *G. assimilis* (mean adjusted hemolymph JHE activity in this base population was 31.3 ± 0.45 nmol/min/ml hemolymph; see below for the meaning of adjusted JHE activity). Each of the three high lines was established by setting up 34 pair crosses each of which consisted of a male and female chosen randomly from the subset of crickets with higher than median JHE activity. Three low lines were initiated in a similar manner using crickets with lower than median enzyme activity and three control lines were set up from the total population of crickets.

Each generation, hemolymph JHE activity was measured in ~100 male and 100 female crickets from each of the three high and three low lines (except for generation 1 where 160 individuals were measured). Enzyme activity was also measured on 20 male and 20 female crickets from each control line each generation. To reduce inbreeding resulting from the differential contribution of selected parents to the progeny pool, selected adults were bred in pairs (randomly chosen) and approximately equal numbers of progeny were taken from each pair cross to produce the next generation. Twenty-five to 30 pairs were set up each generation using crickets with either the highest (high lines) or lowest (low lines) JHE activities or randomly drawn from the line (control lines). Hatchlings were pooled and distributed into a series of plastic boxes and reared as described above except for the last stadium (see below). Overall, 26.7 ± 0.9 (high lines) or 26.0 ± 1.1 (low lines) of the 25–30 crosses produced a sufficient number of offspring to be used. Selection differentials were calculated on selected parents that actually contributed offspring to the next generation.

In the selection experiment, crickets were reared singly in 500-ml plastic containers during the last stadium. This was done to measure the duration of the stadium to correct for its effect on JHE activity (see below). Penultimate-stage crickets were examined for newly molted last-stadium crickets every 24 hr. Newly molted individuals were placed in a 500-ml container with a piece of wet food that was changed every 2 days. Crickets were reared individually until adult molt. Duration of the last stadium typically varied from 9 to 11 days (modal duration over all generations in all lines = 10 days). JHE activity measured on day 3 was negatively correlated with duration of the stadium (*e.g.*, typically 1.3–1.4-fold higher in 9- *vs.* 10-day duration individuals). This negative correlation results from the developmental profile of JHE activity in which activity increases ~10-fold during the first half of the stadium (GU and ZERA 1994). A cricket whose stadium duration is shorter than average has passed through a greater proportion of the stadium when sampled on day 3, relative to an average individual. Hence, its JHE activity is closer to its peak value. To correct for this effect of stadium duration on day 3 JHE activity, adjusted enzyme activities (JHE*adj*) were computed as follows: $JHE_{adj} = JHE_i(JHE_{10}/JHE_i)$ where JHE_i is the median day 3 JHE activity in crickets with a last stadium duration of i days and JHE_{10} is the median day 3 JHE activity in crickets exhibiting a 10-day last stadium. These adjustments were made separately for each sex and normalized all day 3 JHE activities to a physiological age of 0.3 (*i.e.*, the age at which 30% of the stadium had passed).

TABLE 1

Realized heritabilities (±SE) for hemolymph JHE activity

Block	High line	Low line
1	0.258[a]	0.352
2	0.163	0.392
3	*0.242*	*0.175*
Average	0.221 ± 0.029[b]	0.306 ± 0.067[b]
Overall average	0.264 ± 0.038[c]	

Heritabitities were in lines selected for increased or decreased enzyme activity on the third day of the last stadium. JHE, juvenile hormone esterase.

[a] See Figure 1 for heritability estimates in each line.

[b] Heritability estimates do not differ significantly between high and low lines (ANOVA, $F = 1.37$; 1, 5 d.f.; $P > 0.1$).

[c] Average heritability for all six selected lines.

Estimation of heritabilities: Realized heritabilities were estimated by linear regression of mean JHE activity for each generation (cumulative response) on the cumulative selection differential (FALCONER 1989). Because generation means computed from untransformed activities were correlated with sample variances, regressions were performed on means derived from log-transformed activities (FALCONER 1989). Before regression analyses, means of the total and selected populations were corrected for environmental fluctuations each generation by subtraction of unselected control means. Regressions were performed separately on each of the high and low lines of each block. Average realized heritabilities for upward or downward selection were obtained by averaging the heritability estimates (slopes) for the high or low lines across blocks. The standard errors of the average heritabilities are the empirical standard errors derived from the variance in heritabilities among blocks (HILL 1971).

Correlated response to selection on JHE: Hemolymph JHE activities were also measured on days 1, 5 and 8 of the last stadium and on days 2 and 6 of adults during the fifth generation of selection. This was done to estimate correlated responses to selection (realized genetic correlations) between JHE activity on these days with enzyme activity on day 3 of the last stadium. These juvenile ages were sampled because they spanned the age range of the 10-day last stadium. Adults were sampled on the aforementioned ages to obtain individuals before (day 2) or during (day 6) the reproductive period. Hemolymph collection and enzyme assays were the same as for day 3 hemolymph samples. Because a much smaller number of crickets was assayed on days other than day 3, JHE activities were not corrected for stadium duration. Corrected *means* differ from uncorrected means on average by <7%.

Measurement of hemolymph JHE activity and protein concentration: Hemolymph juvenile hormone esterase activity was determined by the radiochemical assay of HAMMOCK and SPARKS (1977) as previously described (ZERA and TIEBEL 1989; GU and ZERA 1994). Activities were measured on diluted hemolymph. Hemolymph protein concentration was measured on an aliquot of whole hemolymph diluted 1/30 with 0.1 N NaOH using the bicinchoninic acid assay (STOSCHEK 1990).

Measurement of whole-cricket JHE and JH-epoxide hydrolase activity: Whole-cricket JHE and JH-epoxide hydrolase activity (which does not occur in the hemolymph, GU and

ZERA 1994), were measured during the fifth (block 3) or sixth (blocks 1 and 2) generations of selection. This was done to determine whether selection on hemolymph JHE activity resulted in corresponding changes in whole-cricket JHE activity as well as correlated changes in JH-epoxide hydrolase activity. JHE and JH-epoxide hydrolase activity were simultaneously measured using the assay of SHARE and ROE (1988). Crickets were homogenized in 5 volumes of 0.1 M phosphate buffer and centrifuged at 6000 × *g* for 15 min. Homogenates were further diluted fivefold with phosphate buffer and were assayed within a few hours. Preliminary experiments identified assay conditions under which product formation was linearly related to time or homogenate (enzyme) concentration.

Whole-cricket *in vivo* JH degradation and excretion: *In vivo* JH degradation was measured to determine if differences in hemolymph JHE activity in the selected lines resulted in corresponding differences in *in vivo* JH metabolism. Experiments were performed during the fifth (blocks 2 and 3) or sixth (block 1) generations of selection. Methods were essentially identical to those used previously by ZERA and HOLTMEIER (1992) on the congener, *G. rubens*. Briefly, ~230,000 DPM of racemic JH-III (= 9 pmol), dissolved in corn oil, were injected into the abdominal hemocoel. A pilot study indicated that ~50% of injected hormone was degraded within 1 hr, and this time period was used in all experiments. After the incubation period, crickets were homogenized in ethyl acetate and the amount of JH-III not degraded was determined by thin layer chromatography and liquid scintillation counting.

The amount of radiolabeled JH and metabolites excreted during the incubation period was determined as described in ZERA and HOLTMEIER (1992). Briefly, after the 1-hr incubation period the posterior portion of each cricket was thoroughly wiped with a tissue moistened with alcohol. The kimwipe was placed in the test tube in which the cricket had been incubated, 1 ml of ethyl acetate was added, tubes were vortexed and an aliquot of ethyl acetate was subjected to liquid scintillation counting. The amount of radioactivity in the extract was determined by liquid scintillation counting.

RESULTS

Direct and indirect responses in hemolymph JHE activity: Approximately 9000 individually reared crickets were scored for day 3 hemolymph JHE activity during the six-generation selection experiment. Activities in the lines typically differed significantly from controls by the second or third generation of selection. By the end of the sixth generation, the high lines exhibited an average 3.5-fold higher hemolymph JHE activity relative to the low lines. Linear regression of response to selection on the cumulative selection differential yielded a nonzero slope for each of the six selected lines (Figure 1; Table 1). Average (±SE) heritabilities, computed by averaging these slopes across blocks, yielded values that were significantly different from zero for either upward ($h^2 = 0.22 \pm 0.03$) or downward ($h^2 = 0.31 \pm 0.07$) selection (ANOVA; $P < 0.025$ in each case). These two heritabilities did not differ significantly from each other (ANOVA; $F_{(1,4)} = 1.37$; $P > 0.1$) indicating no asymmetrical response to selection.

Whole-cricket JHE activity, measured on the third day of the last stadium during generations 5 or 6, was significantly higher in the high line relative to the corresponding low line in each of the three blocks (Table 2). Averaged over the three blocks, whole-cricket JHE activity was 2.0 ± 0.1-fold higher in high *vs.* low lines. This value is significantly lower than the 3.3 ± 0.23-fold difference in hemolymph JHE activity between high and low lines on day 3 during these generations (ANOVA; $F_{(1,4)} = 26.79$; $P < 0.005$).

Correlated responses to selection: Correlated responses to selection on hemolymph JHE activity, measured on days 1, 5 and 8 of the last stadium and days 2 and 6 of adults during generation 5 are presented in Figure 2. Substantial correlated responses were observed during the juvenile stage but not during the adult stage. On each of the 3 days of the last stadium in each of the three blocks, hemolymph JHE activity was significantly higher in crickets of the high *vs.* low line (Kruskal-Wallis tests; $P < 0.005$ in each case; Figure 2). Averaged over blocks, JHE activity was 2.0 ± 0.46, 2.4 ± 0.12 and 2.3 ± 0.29 times as high in the high *vs.* low lines on days 1, 5 and 8, respectively. These differences were only slightly less than the 2.6 ± 0.28-fold activity increase between lines on day 3 (generation 5), the age at which hemolymph JHE activity was selected. Fold increase in JHE activity in high *vs.* low lines did not differ significantly among these age classes (ANOVA; $F_{(3,8)} = 0.64$; $P > 0.1$).

In contrast to the marked indirect responses to selection seen during the juvenile stage, most (four of six) comparisons of JHE activities between the high and low lines in day 2 or day 6 adults were nonsignificant (Kruskal-Wallis tests; $P > 0.05$; see Figure 2). In the two cases where lines differed significantly (block 2, day 2; block 3, day 6), differences were not large (1.1-fold averaged over all blocks and ages) and were not in the same direction in both blocks.

Whole-cricket activity of JH-epoxide hydrolase, the other major JH-degrading enzyme in insects, and hemolymph protein concentration exhibited only weak or undetectable correlated responses in day 3 crickets. JH-epoxide hydrolase differed only marginally (block 1) or not at all (blocks 2 and 3) between the high and low lines (Table 2). Average protein concentration (microgram/microliter hemolymph, ± SE) for the high (H) *vs.* low (L) lines were as follows: BK-1, 41.2 ± 2.4 (H) *vs.* 35.4 ± 1.9 (L); BK-2, 43.8 ± 2.2 (H) *vs.* 45.1 ± 4.8 (L); BK-3, 39.6 ± 1.5 (H) *vs.* 37.6 ± 1.6 (L). Protein concentration differed significantly between the high and low lines in block 1 ($P < 0.05$) but not in blocks 2 or 3 (Kruskal-Wallis tests). Samples sizes ranged from 9–13 per line per block except for the block 1 high line where $n = 6$. Protein concentration did not differ between the sexes in any line.

***In vivo* JH degradation and excretion:** In contrast to the consistent differences or lack of differences between high and low lines with respect to hemolymph and whole-cricket JHE and JH-epoxide hydrolase activity, variable results were observed with respect to *in vivo*

TABLE 2

Whole-cricket JHE and JHEH activity in the high- and low-selected lines

Block	Enzyme	Activity		Results of Kruskal-Wallis test[a]
		High	Low	
Block 1	JHE	11.7 ± 0.9[b] (50.9%)[c]	5.6 ± 0.7 (37.7%)	H = 12.2***
	JHEH	11.3 ± 0.5	9.2 ± 1.1	H = 4.00*
Block 2	JHE	12.2 ± 1.2 (51.8%)	6.1 ± 0.9 (40.0%)	H = 8.99***
	JHEH	11.3 ± 1.1	9.4 ± 1.3	H = 0.80, NS
Block 3	JHE	9.8 ± 1.2 (47.2%)	5.5 ± 0.7 (34.6%)	H = 6.96**
	JHEH	11.0 ± 0.7	10.3 ± 0.9	H = 0.81, NS

Activity was in nanomole JH acid or diol/min/mg wet weight. JHEH, juvenile hormone-epoxide hydrolase.

[a] Comparisons of high *vs.* low activities by the Kruskal-Wallis test (1 d.f.). ***, **, * and NS refer to probabilities <0.005, 0.01, 0.05 and >0.1, respectively.

[b] Values are means ± SE; samples sizes were nine, eight and 12 individuals for block 1, 2 and 3 crickets, respectively.

[c] Percentage of combined JHE and JHEH activity due to JHE. Experiments were performed on crickets from generations 5 or 6 (see MATERIALS AND METHODS).

JH degradation. Percentage JH degraded *in vivo* during the 1 hr incubation period for crickets of the high and low lines of the three blocks are presented in Figure 3 and means are given in Table 3. Percentage JH degraded was significantly greater in the high *vs.* the low line of block 2 (Kruskal-Wallis test; H = 6.87; $P < 0.01$; Table 3) while no differences in JH degradation were observed between the high *vs.* low lines of the other two blocks (Table 3). In each of these experiments, JHE activity was measured on a small (2 μl) blood sample taken from each cricket just before injection of radiolabeled hormone. JHE activity was significantly higher in crickets of the high *vs.* low line in each block (Kruskal-Wallis tests; $P < 0.005$ in each test), irrespective of whether lines differed in *in vivo* JH degradation. However, analysis of these data also indicated that the high line with the highest absolute level of JHE activity (block 2) was the same line that diverged significantly from its corresponding low line with respect to *in vivo* JH degradation. The three low lines were statistically homogeneous with respect to both hemolymph JHE activity and *in vivo* JH degradation (Kruskal-Wallis tests; $P > 0.1$ in all cases; compare means in Table 3). In contrast, the high lines differed significantly among themselves with respect to each of these factors [Kruskal-Wallis tests; $P < 0.005$ (JH degradation) and $P < 0.0.025$ (JHE activity)]. Pairwise comparisons indicated that both JHE activity and *in vivo* JH degradation differed between the high line of block 2 and the high lines of each of the other blocks but not between the high lines of blocks 1 and 3. (Bonferoni-adjusted probabilities derived from Kruskal-Wallis tests were <0.05 for comparisons involving the block 2 high line and high lines from each of the other two blocks. Adjusted probabilities were >0.1 for comparisons between blocks 1 and 3).

In each of the three high and three low lines, the median amount of hormone and metabolites excreted during the 1 hr incubation period was <3% of amount of radiolabel injected (n = 9–12 determinations per line per block). The amount of excreted radiolabel did not differ significantly between high and low lines of any of the three blocks (Kruskal-Wallis tests, $P > 0.1$ in all comparisons).

DISCUSSION

Direct response to selection and heritability: To our knowledge, the present study represents the first quantitative-genetic analysis of naturally occurring endocrine variation in an insect species. Results have important implications for the microeveolution of the endocrine factors themselves as well as traits which are regulated by these factors.

The significant realized heritability for day 3 hemolymph JHE activity ($h^2 = 0.26 \pm 0.04$; Table 1) indicates that this endocrine character has sufficient additive genetic variance to allow rapid evolutionary change. This heritability estimate does not differ significantly from a preliminary estimate of h^2 for JHE activity during the early last stadium measured on an independently derived laboratory population of *G. assimilis* ($h^2 = 0.57$ pm 0.27; GU and ZERA 1996). The JHE heritability is also similar to those for enzymes of intermediary metabolism in Drosophila (CLARK 1990) suggesting that heritabilities of insect endocrine traits may be no different from those of "housekeeping" enzymes. Recent quantitative-genetic studies of physiological traits have also documented significant heritabilites for both organismal performance (*e.g.*, speed and endurance) and enzyme activities (reviewed in GARLAND 1994).

The nature of the hemolymph JHE activity differ-

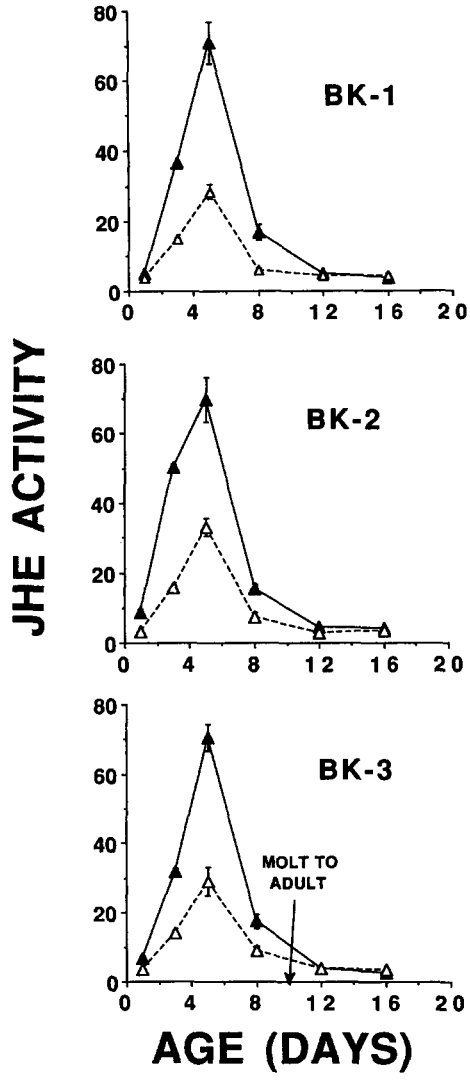

FIGURE 2.—Correlated responses to selection on day 3 hemolymph JHE activity in *G. assimilis*. Symbols are the same as those in Figure 1. Age represents days from molt to last juvenile stadium. Median duration of the last stadium was 10 days; days 12 and 16 represent days 2 and 6 of adults. JHE activity = nmol/min/ml hemolymph.

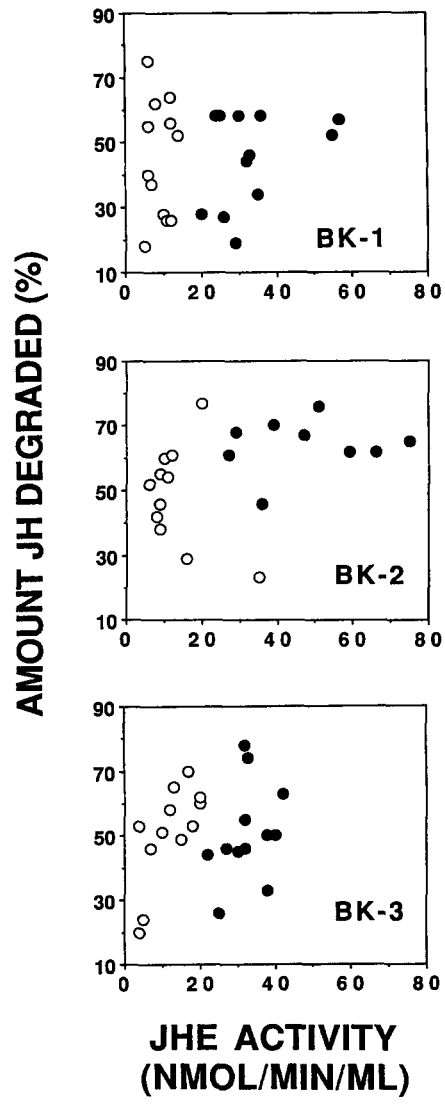

FIGURE 3.—Percentage JH degraded *in vivo* as a function of hemolymph JHE activity in high (●) and low (○) selected lines of *G. assimilis*. Each point represents the measurement of *in vivo* JH degradation and hemolymph JHE activity from a single cricket. JHE activity was measured on a small hemolymph sample taken just before injection of radiolabeled hormone. BK, block (see MATERIALS AND METHODS).

ences between the high-and low-selected lines of *G. assimilis* on day 3 of the last stadium is currently unknown. It could be due to a variety of factors such as variation in kinetic properties of allozymes or regulatory factors that affect the synthesis, degradation or tissue localization of JHE activity. We are in the process of biochemically characterizing the JHEs from the high and low selected lines to investigate this issue. Whatever factors underlie the response to selection on hemolymph JHE activity they appear to be fairly specific to JHE. Neither hemolymph protein concentration nor whole-cricket JH-epoxide hydrolase activity (which does not occur in the hemolymph) diverged appreciably between the high and low selected lines of any block (RESULTS; Table 2).

In the analogous situation of JHE activity variation in the wing-polymorphic congener, *G. rubens*, no differences were observed between JHEs from the high and low activity genetic stocks with respect to thermostability, Michaelis constant or inhibition by a variety of compounds (ZERA *et al.* 1992). This implicates selection on regulatory factors as the underlying cause of the JHE activity differences between stocks of *G. rubens*. Variation at regulatory loci accounts for a large proportion of the genetic variation in enzyme activity for enzymes involved in intermediary metabolism in *Drosophila melanogaster* (LAURIE-AHLBERG *et al.* 1980, 1982).

Whole-cricket JHE activity was significantly elevated in the high *vs.* low line in each of the three blocks (Table 2). However, the average fold increase of enzyme activity in the high lines was significantly less for

TABLE 3

In vivo* juvenile hormone degradation and *in vitro* hemolymph JHE activity in high- and low-selected lines of *Gryllus assimilis

		Line		
	Measurement	High	Low	Results of K-W Test[a]
Block 1	JH-DEG[b]	44.9 ± 4.2	44.9 ± 5.3	$H = 0.05$; $P > 0.1$
	JHE-ACT[c]	33.4 ± 3.3	9.1 ± 0.9	$H = 17.3$; $P < 0.005$
Block 2	JH-DEG	64.1 ± 2.8	48.8 ± 4.6	$H = 6.87$; $P < 0.01$
	JHE-ACT	47.7 + 5.5	13.2 + 2.5	$H = 7.30$; $P < 0.01$
Block 3	JH-DEG	50.8 ± 4.4	50.9 ± 4.4	$H = 0.65$; $P > 0.1$
	JHE-ACT	32.6 ± 1.8	12.1 ± 1.8	$H = 17.3$; $P < 0.005$

[a] Results of Kruskal-Wallis tests (1 df).

[b] Percentage radiolabeled juvenile hormone degraded within 1 h after injection; values are means ± SE of 12 replicates except for block 2 ($n = 9$ and $n = 11$ for the high and low lines, respectively).

[c] nmol JH acid/min/ml hemolymph; activities were measured on the same individuals in which *in vivo* JH degradation was measured just before hormone injection. Individual values for JHE activity and percent JH degraded *in vivo* are presented in Figure 3. Measurements were performed on crickets from generations 5 or 6.

whole-cricket activity (2.0 ± 0.1-fold) compared with hemolymph activity (3.3 ± 0.3-fold; see RESULTS). This indicates that the divergent hemolymph JHE activities likely resulted from the selection of segregating genetic factors regulating whole-organism enzyme activity as well as factors that regulate the partitioning of enzyme into the hemolymph *vs.* other tissues. Note that this explanation is contingent upon the absence of differences in hemolymph volume between the high and low lines. We recently documented that hemolymph volume does not differ between high and low lines of any of the three blocks on day 3 of the last stadium (A. ZERA, J. SALL and R. SCHWARTZ, unpublished observations). Hemolymph volumes also do not differ between high and low JHE activity lines in the closely related *G. rubens* (ZERA and HOLTMEIER 1992). An increasing number of studies are focusing on genetic variation in the tissue expression of various enzymes as models of the evolution of gene regulation (DICKINSON 1975, 1988; PAIGEN 1979; HAMMER and WILSON 1987; BUSH and PAIGEN 1992). We are currently quantifying the activities of JHE and JH-epoxide hydrolase, the other major enzyme involved in *in vivo* JH degradation (HAMMOCK 1985; ROE and VENKATESH 1990), in various tissues to obtain more direct information on genetically variable tissue-specific regulators of these enzymes in the selected lines.

Genetic correlations: The extent to which individual endocrine traits are free to evolve independently *vs.* constrained to evolve in concert with other hormonal features is an important issue in the evolution of the insect endocrine system. The strength of genetic correlations between traits will determine the degree to which these alternatives prevail. There is currently no published information on genetic correlations between endocrine factors in insects, even though various evolutionary scenarios have been postulated that require the existence of such correlations (*e.g.*, the evolution of flightlessness; FAIRBAIRN and ROFF 1990; discussed below).

One of most important findings of the present study was the strong indirect responses to selection for hemolymph JHE activity on days 1, 5 and 8 (Figure 2). These indirect responses document the existence of strong genetic correlations between JHE activity on day 3 and each of these other days of the last stadium. The ratio of enzyme activity between the high and low lines averaged over blocks is statistically indistinguishable among each of the 4 days of the last stadium (see RESULTS). Thus, selection on day 3 of this stadium altered hemolymph JHE activity to the same degree on days 1, 5 and 8 as on the day selected. That is, the magnitude of the entire JHE developmental profile during the last stadium evolved while the shape of the profile remained constant. We observed no variation for genetic factors that alter the shape of the JHE developmental profile during the last stadium (*e.g.*, variable temporal loci *sensu*; PAIGEN 1979).

Although not measured directly, hemolymph JHE activity on days 1, 5 and 8 must also have significant heritabilities. This is so because an indirect response to selection requires nonzero heritabilities for both the selected and correlated traits (FALCONER 1989). Significant heritabilities were also found by GU and ZERA (1996) for hemolymph JHE activity during the early (days 2–3), middle (days 4–6) but not late (days 7–9) portions of the last stadium of an independently derived population of *G. assimilis*.

In contrast to the strong indirect responses to selection on JHE activity observed on days 1, 5 and 8 of the last juvenile stadium, we found no significant indirect responses on either day 2 or day 6 of adulthood (Figure 2). This result could conceivably be due to a zero heritability for JHE activity on these adult days and/or the absence of a genetic correlation between JHE activity on days 2 or 6 in adults and day 3 in juveniles (see above). We recently documented a significant response to selection on hemolymph JHE activity in day-6 adult

G. assimilis (= nonzero heritability) in a separate selection experiment (A. J. ZERA, unpublished data). The adults used in that experiment were taken from the same base population from which individuals were obtained for the present study. Thus, the lack of an indirect response to selection in day-6 adults must be due to the absence of a genetic correlation.

The lack of indirect responses in adults is noteworthy. It indicates that, at least in the short term, JHE activity in juveniles can evolve independently from enzyme activity in adults. That is, endocrine variation affecting development is decoupled from variation affecting reproduction. Note that this conclusion only applies to day 3 of the last stadium and days 2 and 6 of adults. Because inferences concerning genetic correlations can only be made between a direct and an indirect response to selection (FALCONER 1989), it is unknown whether genetic correlations exist between JHE activities during adult stages and days 1, 5 or 8 of the last stadium.

The absence of an indirect response to selection on whole-cricket JHEH activity is also noteworthy (Table 2). JHEH is the other major JH-degrading enzyme (or group of enzymes) and a lack of a genetic correlation between JHE and JHEH activities indicates that these two degradative systems can evolve independently. On the other hand, we have recently documented strong positive genetic correlations between JHE and JH binding activity throughout the last stadium (A. J. ZERA and R. SCHWARTZ, unpublished data). The significance of correlations between some important factors that regulate the JH titer (JHE and JH binders) but no measurable correlations between others (JHE and JHEH) is unclear.

There are currently no other comparable developmental-genetic data on endocrine correlations within or between stages in either *G. assimilis* or other insects. Thus we cannot assess the generality of the pattern that we observed in the present study. Our results are similar in some respects to the extensive quantitative genetic analyses of developmental variation of morphological traits in rats and mice (ATCHLEY and RUTLEDGE 1980; CHEVERUD *et al.* 1983). When the same trait (*e.g.*, tail length) was measured at several points in development, correlations between traits were the highest for the closest ages and the magnitudes of the correlations dropped steadily between increasingly distant ages. In the present study, however, temporal variation in JHE correlations was much more of a step function rather than a gradual decrease.

The JHE activity developmental profiles in selected lines of *G. assimilis* are very similar to profiles observed previously in stocks of the congener *G. rubens* (ZERA and TIEBEL 1989; ZERA *et al.* 1993). Long-winged and short-winged lines of *G. rubens* differed by 2–4-fold in JHE activity during the last stadium while enzyme activities were equivalent during the adult stage. This similarity in JHE developmental profiles bears on the important but poorly understood issue of the long-term stability of genetic correlations (TURELLI 1988). Models of multivariate evolution typically assume constant genetic correlations (*e.g.*, LANDE 1979) while both theoretical (TURELLI 1988) and experimental (WILKINSON *et al.* 1990) studies indicate that selection may change correlations. The similar genetically specified JHE activity developmental profiles in both *G. rubens* and *G. assimilis* raises the possibility that the correlations which comprise these profiles (*i.e.*, the genetic variance-covariance matrix) may have been stable over evolutionary time (*i.e.*, since the divergence of these two species). Assessment of this highly speculative hypothesis requires additional detailed information on genetic correlations between JHE in various ontogenetic stages in both *G. assimilis* and *G. rubens* as well as information on the phylogenetic relationship of these two species.

As mentioned previously, the lack of genetic correlations between adult and juvenile JHE activities has some interesting implications for recent models of the evolution of flightlessness. The most recent model (ROFF 1986; FAIRBAIRN and ROFF 1990) is based on the strong inhibitory action of juvenile hormone (JH) on the development of flight capability (wings and flight muscles) in juveniles and the strong positive effect of this hormone on adult fecundity. The evolution of flightlessness has been postulated to result from a correlated response to selection acting to increase adult fecundity. The increased JH titer in adults, which presumably underlies the increased fecundity, is thought to cause an increased titer in juveniles via an indirect response to selection. The increased titer in juveniles, in turn, blocks the development of wings and flight muscles. This hypothesis accounts for the positive association between flightlessness and elevated egg production found in many wing polymorphic insects (ROFF 1986). However, there is currently no direct evidence on the existence of genetic correlations between the JH titer in adults and juveniles. Results of the present study, where no correlation was observed for an important regulator of the JH titer in adults and juveniles, provide no support for this idea.

JHE activity variation and whole-cricket hormone metabolism: If altered JHE activities are to have any affect on the expression of whole-organism traits, the degree of alteration must be sufficient to change the *in vivo* rate of JH degradation. The magnitude by which JHE activity must be altered to accomplish this is poorly understood (ZERA and HOLTMEIER 1992). This issue is complicated since *in vivo* JH degradation is affected by enzymes other than JHE, most notably JH-epoxide hydrolase (HAMMOCK 1985; ROE and VENKATESH 1990; ZERA *et al.* 1993).

In vivo studies of JH degradation in the selected lines indicate that both the absolute level of JHE activity and the degree of divergence in activity are important in producing line differences in *in vivo* JH degradation.

The greatest divergence in JHE activity occurred in blocks 1 and 2 where a 3.6–3.7-fold elevation in activity was observed between the high *vs.* low line of each block (Table 3). Yet *in vivo* JH degradation differed between high and low lines only in block 2 (Table 3). This indicates that the relative difference in enzyme activity in and of itself is not sufficient to produce measurable differences in *in vivo* JH metabolism between the lines. JHE activity and JH degradation were especially elevated in the high line of block 2 compared with the high lines of the other two blocks (see RESULTS, Table 3 and Figure 3). This suggests that JHE activity must be elevated above some baseline level to have a measurable effect on *in vivo* JH metabolism. An alternate possibility is that some unmeasured factor affects *in vivo* JH metabolism and varies between the blocks.

In summary, we have documented significant genetic variability for at least one important component of the insect endocrine system, juvenile hormone esterase activity. More importantly, we have documented several cases where genetic correlations between various endocrine components during the same or different ontogenetic stages are very strong or very weak. We are currently investigating the biochemical bases of the JHE activity variation between the high- and low-selected lines to better understand the nature of the response to selection.

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