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A Comparative Analysis of Juvenile Hormone Metabolyzing Enzymes in Two Species of *Drosophila* During Development

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The course of changes in the activities of enzymes degrading juvenile hormone (JH), epoxyde hydrolase (JHEH) and JH-esterase (JHE) was studied in two lines of *Drosophila virilis* (101 and 147) and in two lines of *D. melanogaster* (Canton-S and 921283).

It was established for *D. virilis* that changes in the JH titre during pupal-adult development is determined by the activity level of JHE rather than JHEH, while in *D. melanogaster* developmental changes in JH titre are related to changes in the activity level of both JHE and JHEH. In adults of *D. virilis*, the high level of JH-hydrolysing activity is determined by JHE and in those of *D. melanogaster* by JHEH. Differences in the course of changes in the JHE activity level between adults of lines 101 and 147 of *D. virilis* were found, and also in the JHEH activity level between adults of lines Canton S and 921283 of *D. melanogaster*. It was shown that attainment of a definite JHE activity level in females of lines 101 and 147 agrees well with the onset of oviposition of fertilized eggs. The possible role of JHE in reproduction of *D. virilis* is discussed. Copyright © 1996 Elsevier Science Ltd

Juvenile hormone JH-esterase Epoxyde hydrolase *Drosophila* Development

INTRODUCTION

It is known that the juvenile hormone plays a very important role in development and function of insects. It controls passage from one developmental stage to another: larval molt occurs at a very high titre of JH; when JH titre lowers, metamorphosis follows: and in the absence of the hormone molt of the adult takes place (Hammock, 1985; Riddiford, 1985; Nijhout, 1994). In the adult, JH regulates reproductive maturation (Koeppe *et al.*, 1985; Shapiro *et al.*, 1986; Yamamoto *et al.*, 1988), and it is involved in the control of diapause (Delinger and Tanaka, 1989) and caste determination (Rachinsky and Hartfelder, 1990).

Regulation of JH titre is achieved through a balance between synthesis and degradation. The major pathways of JH degradation are hydrolysis of the ether group by JHE and hydratation of the epoxide ring by JHEH (Hammock, 1985). It was shown in some insects that JHE plays the major role in insect development under normal conditions. Using inhibitors it was demonstrated that the dramatic increase in JHE at key developmental times results in the degradation of JH in haemolymph and other tissues before metamorphosis (Hammock, 1985; Rauschenbach, 1991; Cymborowski, 1991).

In the adult JH plays a major role in regulation of reproduction by performing a gonadotropic function. It controls previtellogenin growth of follicles in females and maturation of gonadal tissues. When acting together with 20-hydroxyecdysone, it induces the synthesis of vitellin proteins in fat body. Furthermore, JH precisely controls the uptake of vitellogenins by the oocyte (Jowett and Postlethwait, 1981; Postlethwait and Parker, 1987; Bownes, 1989). However, analysis of the available data on JH titres reveals that gonadotropic effects in various insect species seem to require different levels of JH (De Kort, 1990). The mechanisms of this diversity are as yet unclear; however, it is apparent that the degradation system of JH in adult acquires particular importance in this regard.

Recently we have disclosed that there are two forms of JHE in *D. virilis* (Rauschenbach *et al.*, 1995). The course of changes in the activity of one, the DFP-insensi-

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tive (p-esterase), has been earlier studied in detail and, as a result, we have demonstrated its key role in the development of *D. virilis* during metamorphosis both under normal and stress conditions (Rauschenbach *et al.*, 1991).

Here we present a detailed account of a study of the course of changes in the activities of enzymes degrading JH, JHEH and JHE during development of two lines of *D. virilis* and of two lines of *D. melanogaster*. Their possible role in the regulation of JH titre in critical developmental periods and in reproduction are discussed.

MATERIALS AND METHODS

This study was performed with two lines (101 and 147) of *D. virilis* and two lines of *D. melanogaster*. (Canton-S and 921283). Line 101 is of wild type, line 147 bears the brick, broken and detached on chromosome II and temperature-sensitive lethal on chromosome VI. The two lines of *D. virilis* contrast in their response to heat stress (Rauschenbach *et al.*, 1987, 1995). Canton-S line is wild and line 921283 was derived from an isofemale line from a natural population of *D. melanogaster* of GornoAltaisk (West Siberia) by individual crosses through five successive generations; they also differ in their response to stress (Gruntenko *et al.*, 1995). Because these differences in JH metabolism and response to stress were established only for adult females of these lines (Rauschenbach *et al.*, 1995), our subsequent studies, including the present one, were conducted using females only.

Cultures of both species were synchronized by hatching, puparium formation and fly emergence. Individuals of both species were frozen in liquid nitrogen and stored at −20°C until further use. The experiments were performed in spring.

Measurements of hydrolysis of radioactive JH were carried out by the method of Hammock and Sparks (1977). A fly was homogenized on ice in 30 μl of 0.1 M Na-phosphate buffer, pH 7.4, containing 0.5 mM phenylthiourea. The homogenates were centrifuged for 5 min at 12 000 rpm, and samples of the supernatant were utilized for the reaction.

Aliquot size and reaction time was determined in a separate experiment as described earlier (Rauschenbach *et al.*, 1995). The reaction was performed by either preincubating the supernatant with diisopropylfluorophosphate for 30 min (DFP, final concentration 0.0005 M) or without DFP.

A mixture consisting of 0.1 μg unlabeled JH III (Sigma) and of 12 500 dpm ^{3}H chain labeled JH III (17.4 Ci/mmol at C-10, NEN Research Products) was used as substrate. The reaction was carried out in siliconized tubes in 100 μl of incubation mixture at 37°C.

The products of hydrolysis of labeled JH-III were analyzed by TLC according to Renucci (1986). The reaction products were extracted with ethyl acetate and applied to silica gel plates (60 F 254, Merck). Chromatography was performed with a solvent composed of toluene : ethyl acetate : acetic acid (70:30:1, v/v/v). After chromatography (the length of separation lanes was 14 cm), the plates were dried, the lanes corresponding to each sample were cut into pieces 1 cm in size, placed into vials with a toluene scintillator, and the amount of radioactivity was measured in a Delta-300 counter. In the present experiments acid-diol and diol migrated next to each other (the second and third centimetre on the chromatogram). Thus there was a high probability of a part of the product of getting into the neighbor zone during cutting of the chromatogram. For this reason these zones are combined into one group.

The significance of the differences between the data sets was tested by Student's t-test.

RESULTS

In this manuscript we refer to the JHE activity as any esterase activity acting on JH as a substrate and JHEH as any epoxide hydrolase activity acting on JH as a substrate both under the assay conditions described here. We do not suggest that only one enzyme is involved in each of these catalytic activities, nor do we suggest that it is the same enzyme or group of enzymes in each species and developmental stage. We present data correlating these enzyme activities in whole body homogenates with developmental events. Of course other experiments will be needed to support a hypothesis that the degradation of JH resulting from these activities has a developmental role.

Earlier it was found (Rauschenbach *et al.*, 1995) that the short term stress causes the sharp decrease of JHE activity of wild type flies (in particular, in the flies of the line 101 of *D. virilis* and Canton-S of *D. melanogaster*), and the stress does not affect on JHE activity in the flies of mutant line 147. Also, we established that anaesthesia and process of injection are the stress factors for *Drosophila* (Rauschenbach *et al.*, 1987, 1993). Proceeding from these assumptions, the measurements of enzyme activities were performed on fly homogenates *in vitro*, but not *in vivo*, by ^{3}H-JHIII injection into flies.

Figure 1 presents the results of measurements of the activities of enzymes hydrolyzing JH, JHEH and JHE in *D. virilis* (line 101) during development.

It is seen that JHEH activity (discontinuous line) is high in larvae, falls sharply in prepupae and virtually remains relatively constant during pupal-adult development. Presumably, variations in the activity of this enzyme during pupal-adult development do not play an important role in the regulation of the JH titre in *D. virilis*.

As regards JHE, as already indicated it has been previously shown that two forms of the enzyme are present in *D. virilis*; one DFP-sensitive, the other DFP-insensitive (Rauschenbach *et al.*, 1995). With this in mind, the activity of JHE was determined in the presence and absence of the inhibitor DFP. Figure 1 presents the total

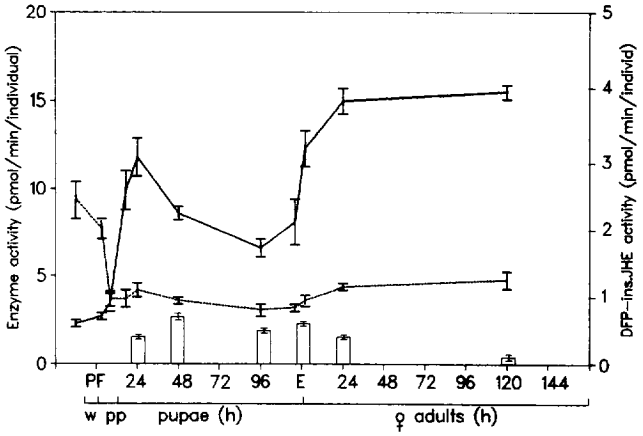

FIGURE 1. The activity of JH metabolyzing enzymes during pupal–adult development of 101 line of *D. virilis*. Continuous line=total JHE activity, discontinuous line=JHEH activity (left scale), columns=DFP-insensitive JHE activity (right scale). *X*-axis=the stages of development (age in hours): PF=puparium formation; E=emergence; w=wandering larvae; pp=prepupae. Each value in the plot is the average of 5–10 TLC assays.

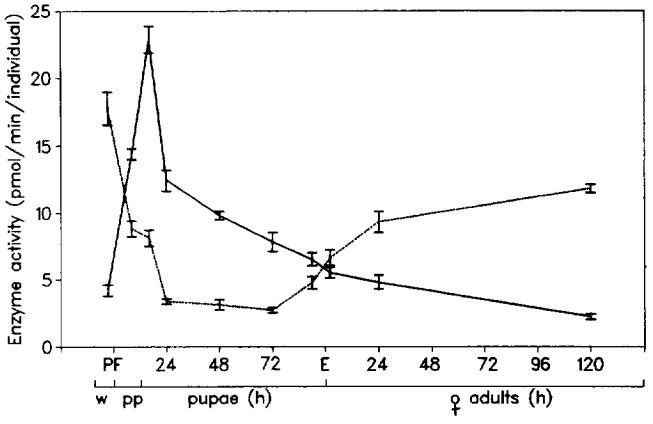

FIGURE 2. The activity of JHE and JHEH during pupal–adult development of *D. melanogaster* (Canton S. line). Continuous line=JHE activity, discontinuous line-JHEH activity. Designations are the same as in Fig. 1.

activities of both forms of JHE (continuous line), and the activity of the DFP-insensitive form of the enzyme is represented in columns. From the results it follows that firstly the activity of DFP-insensitive JHE in the studied developmental periods is one order of magnitude lower than that of DFP-sensitive and, accordingly, its contribution to JH metabolism is lower. Secondly, the course of changes in the activities of these forms of esterases is coincident during the period of pupal development and the period preceding emergence of adult flies. Evidence for this is provided by the observed increase in the activity of both esterases during the developmental periods indicated above when a decrease in JH titre is thought essential for a successful passage through metamorphosis and emergence. Thirdly, the course of changes in the activity of two forms of JHE is reverse after fly emergence. Thus the activity of DFP-insensitive JHE falls after fly emergence, and in 5 day old flies it does not exceed 0.07 pmoles/min/fly, while the activity of DFP-sensitive JHE after emergence continues to rise, and reaches maximum 1 day after fly emergence (exceeding 15 pmoles/min/fly), and virtually does not change until the fifth day after emergence. These data show that at the adult stage DFP-insensitive JHE presumably does not play an important role in JH metabolism, and ester hydrolysis in adults is determined, to a large extent, by the activity level of DFP-sensitive JHE.

Figure 2 shows the measurement data for the activity level of JHE and JHEH in *D. melanogaster* (Canton-S line) during development. It is seen that JHE (continuous line) has a single activity peak during the period of metamorphosis. During subsequent development, the activity of the enzyme rapidly falls and reaches minimum values in adult flies. In contrast to JHE, the activity of JHEH (discontinuous line) falls sharply after pupation, rises again before emergence and remains at a high level in mated females. Thus in *D. melanogaster*, as with *D. virilis*, there are two surges of high JH-hydrolytic activity, they are provided by different enzymes.

Figure 3 compares the data for total JHE activity in flies of lines 101 and 147 of *D. virilis* during pupal–adult development. It is clear that during metamorphosis the course of changes in JHE activity in individuals of line 147 generally coincides with the one of line 101, however, in the 24 h after fly emergence the JHE activity level in individuals of line 147 becomes considerably lower than in those of line 101 ($p>0.999$). Also the level of the enzyme activity, which is observed in individuals of line 101 the next day after emergence, is reached by flies of line 147 only on day 6.

The measurement data for JHEH activity level in flies of lines 147 and 101 of *D. virilis* during development are compared in Table 1. As the table shows, during metamorphosis, fly emergence and in mated adults of line 147, the activity of the enzyme is close to the one in individuals of line 101. This allows us to suggest that in flies of line 147, as with those of line 101, JHEH does

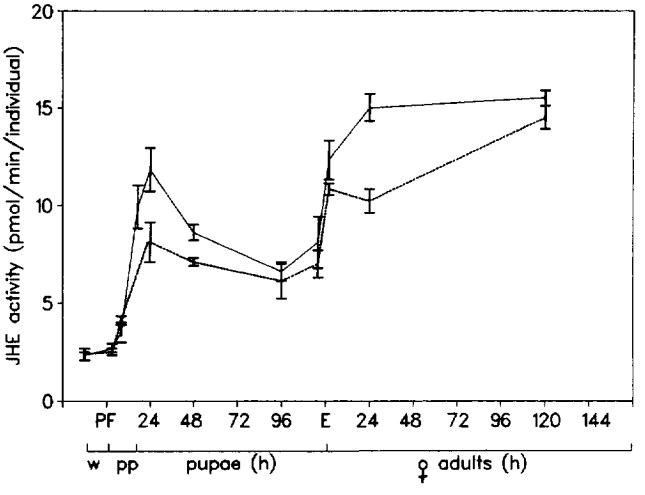

FIGURE 3. JHE activity during development in lines 101 and 147 of *D. virilis*. Continuous line=line 101; discontinuous=line 147. Designations are the same as in Fig. 1.

TABLE 1. JHEH activity during development of lines 101 and 147 of *D. virilis*

Stages of development (age in hours)	JHEH activity (pmol/min/individual)	
	Line 101	Line 147
Wandering larva	9.4±1.0	8.9±0.2
Prepupae		
2 h	7.7±0.6	-
8 h	3.7±0.4	3.0±0.3
Pupae:		
24 h	4.2±0.4	3.2±0.6
48 h	3.6±0.2	3.0±0.2
118 h	3.1±0.3	2.9±0.2
Females:		
1 h	3.6±0.3	3.2±0.4
24 h	4.4±0.2	4.3±0.3
120 h	4.8±0.5	4.5±0.2

—=not measured.

not play as significant role in developmental regulation of JH titre as JHE. It must be remembered, however, that many *in vitro* measurments are not predictive of *in vivo* pharmacodynamics. Also, whole body homogenates obscure the tissue distribution of enzyme activities, which could result in important local changes in JH metabolism.

Figure 4 presents data on JHE activity level during development in line 921283 (discontinuous line) of *D. melanogaster* compared to that in Canton-S line (continuous line). It is apparent that the course of changes in the activity of the enzyme in individuals of 921283 line is similar to the one in flies of Canton-S, however, the activity level of the enzyme is lower in the former during almost all the studied periods ($p>0.999$ for prepupae and for 16, 24 and 48 h pupae; $p>0.95$ for 72 and 92 h pupae; $p>0.99$ for the fly emergence; $p>0.999$ for 24 h adults), except for wandering larvae and mated females. As for JHEH, as shown in Fig. 5 its temporal changes in activity are very similar in the two lines during the period of metamorphosis. After fly emergence, JHEH activity in flies of line 921283 is appreciably lower than in flies of Canton-S line ($p>0.999$), i.e. line 921283 is generally characterized by a lower level of JH-hydrolysis throughout all the studied period. However, if during metamorphosis it is determined by a lower activity level of JHE, and it is related to low JHEH activity in mated females.

Proceeding from the role of JH in insect reproduction, we suggested that differences in JHE activity level between females of lines 101 and 147 may have an influence on their reproductive performance.

The data for reproduction of lines 101 and 147 of *D. virilis* are given in Table 2. It is seen that these lines differ, firstly, in the onset of oviposition of fertilized eggs. Females of line 101 start oviposition of fertilized eggs 2 days after emergence and females of line 147 only 7 days after it. Secondly, they oviposit different numbers of eggs (calculated per female per day): females of line 101 oviposit three times more eggs than females of line 147. Thirdly, lines 101 and 147 differ in viability of individuals at the pupal stage. As a result, the number of emerged flies (calculated per female per day) in line 147 is appreciably lower than in 101.

Having suggested that later onset of oviposition flies of line 147 is related to low level of JHE activity during the first days after fly emergence, we injected females of line 147 JHE of *Heliothis virescense*. The isolation procedure of JHE of *H. virescense* and its purification have been previously described (Shiotsuki *et al.*, 1994). Two days after fly emergence, females were injected with 0.5 μl JHE deluted in Beadle's medium so that activity of enzyme would be 15 pmol/min/ul. Control flies were injected with 0.5 μl of Beadle's medium. After the injection, females were placed into vials with food (three males and three females per vials), flies were transferred to fresh food daily and the number of eggs laid each

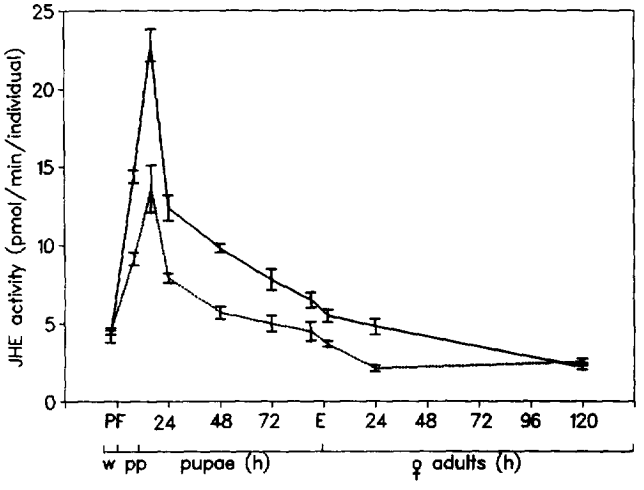

FIGURE 4. JHE activity during development in lines Canton S and 921283 of *D. melanogaster*. Continuous line=Canton-S, discontinuous=line 921283. Designations are the same as in Fig. 1.

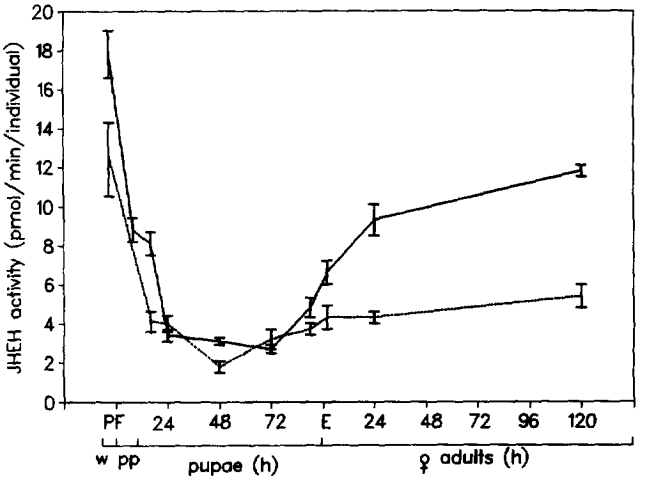

FIGURE 5. JHEH activity during development in lines Canton S and 921283 of *D. melanogaster*. Continuous line=Canton-S, discontinuous=line 921283. Designations are the same as in Fig. 1.

TABLE 2. Comparative reproduction characteristics in lines 101 and 147 of *D. virilis*

Line	A number of parent pairs	Start of oviposition of fertilized eggs (days after emergence)	Quantity of ovipositing eggs during day per female	Viability during pupal development (%)	Quantity of emerged flies during day/female
101	60	2	16.2±1.4	96.9±1.1	12.6±0.3
147	63	7	5.0±1.9	70.6±4.8	1.0±0.1

Quantity of ovipositing eggs and, respectively, quantity of emerged flies were calculated during the days after the start of the oviposition of fertilized eggs. Viability during pupal development was determined as the ratio of the number of flies, emerged in vial to one pupae in this vial.

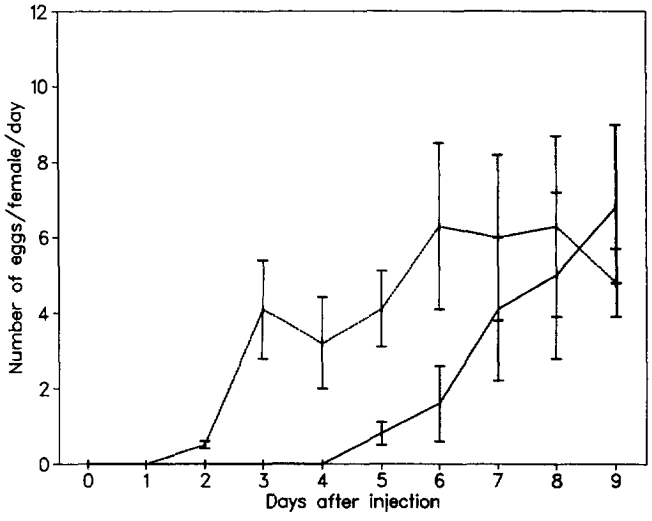

FIGURE 6. The influence of JHE injection on oviposition onset in females of line 147 of *D. virilis*. Continuous line=control females, discontinuous=females injected with JHE of *Heliothis virescense*.

day was counted. JHE was injected into 30 females and Beadle's medium into 25 females.

The experimental results are summarized in Fig. 6 and Table 3. From the data it follows that on the second day after injection JHE-treated females start laying eggs, whereas the controls start to do so on the fifth. However, it should be noted that not all females start to lay eggs after JHE injection. Thus, of the eight vials with the treated flies, in two vials flies started to lay eggs almost at the same time as controls. Presumably, this was because in all females the level of injected JHE was not sufficient to produce an increase in JH metabolism to a level needed to provide oviposition onset. In fact, measurement of JHE activity in females 2 h after injection demonstrated that it varied from 11.2 to 17.3 pmol/min/fly in JHE-treated flies; in contrast the variation was from 9.6 to 12.3 pmol/min/fly in the controls.

TABLE 3. Effect of injection of JHE of *H. virescence* on JH metabolism in females of line 147 of *D. virilis* (the results of TLC assays)

Group	Enzyme activity (pmol/min/fly) JHEH	JHE
Control	4.0±0.2	11.1±0.5
JHE treated females	4.3±0.2	15.5±0.5

DISCUSSION

Campbell *et al.* (1992) have presented a developmental profile of JH-hydrolytic activity in *D. melanogaster*. Two enzymes hydrolyzing JH, JHE and JHEH were identified. The authors observed a single peak of JH-hydrolytic activity during pupal-imaginal development of *D. melanogaster*. The peak is related to metamorphosis, and it is, to a large extent, determined by the activity of JHE. Campbell *et al.* (1992) have noted a considerably lower level of JH-hydrolytic activity in mated females compared to pupae, which, as may be judged from their data, is mainly determined in females by the activity of JHEH. Comparison of these results with the results of JH titre in *D. melanogaster* reported by Bownes and Rembold (1987) provide evidence indicating that JH-hydrolyzing activity in the course of development of *D. melanogaster* is a faithful reflection of JH titre. However, it should be stipulated that the measurement data for JH titre in *D. melanogaster* (Bownes and Rembold, 1987) are expressed per individual, while Campbell *et al.* (1992) have determined JH-hydrolyzing activity on the basis of mg of protein. At the same time as Bownes and Rembold (1987) have noted, body weight (and protein content) in adult flies includes the weight of developing oocytes in which JH has not been identified. For this reason we believe that JH-hydrolytic activity, when expressed per mg of protein, introduces some distortion into the real pattern of interaction between the system of synthesis and degradation of JH in mated females of *D. melanogaster*.

In fact, as follows from our data, the difference in the level of JH-hydrolyzing activities between pupae and adults of *D. melanogaster*, when expressed per individual, are not so striking (see Fig. 2) as follows from the study of Campbell *et al.* (1992), and it may be said that there are two surges in JH-hydrolyzing activity in *D. melanogaster*. The second surge appears soon after the fly emergence, and it is determined by a high level of JHEH activity. It is pertinent to note that this is in agreement with Bownes (1989) who has demonstrated that JH content in 24 h females of *D. melanogaster* is 2.5-fold lower than in freshly emerged flies.

Thus, a high level of JH-hydrolyzing activity is observed in the homogenates of adults of *D. melanogaster*. It is comparable to the one in JH metabolism in pupae, although, as follows from the results of Bownes

and Rembold (1987), JH titre is significantly higher in adults than in pupae. Bearing this in mind, we believe that while during metamorphosis developmental changes in JH titre in *D. melanogaster* are greatly influenced by alterations in the activity level of enzymes degrading JH, adults changes in the JH titre are influenced more by changes in biosynthesis against a higher, rather uniform background of degradation of the hormone. From this hypothesis we would predict higher basal levels of JH production in adults at key times in the reproductive cycle to overcome degradation. This hypothesis appears reasonable as will be discussed below.

It should be noted that in *D. virilis* JH is actually hydrolyzed by three enzymes—JHEH and two forms of JHE differing in their sensitivity to the inhibitor DFP. In contrast to *D. melanogaster*, in *D. virilis* JHEH activity changes little, if at all, in the course of pupal-imaginal development and therefore JHEH presumably does not play an important role in the regulation of JH titre.

Regarding the two forms of JHE, as indicated above, the level of their activities changes synchroniously during larval–pupal development up to adult emergence, and the profile of activity resembles the one for JHE in *D. melanogaster*. The high level of JHE activity during pupal development in *Drosophila* supports our previous data, indicating that JHE plays a key role during passage through metamorphosis in *D. virilis* (Rauschenbach *et al.*, 1991), and this is also in agreement with the results obtained with other insect species (Hammock, 1985; Riddiford, 1985; Nijhout, 1994). After emergence, the activity of DFP-insensitive JHE falls in females, so the developmental profile of activity of this JHE form in *D. virilis* coincides with the one in *D. melanogaster*.

The activity of the second form of the enzyme sharply increases at the beginning of the adult stage and afterwards it virtually remains unaltered, i.e. in *D. virilis*, in contrast to *D. melanogaster* a high level of JH-hydrolyzing activity in mated females is determined by JHE in its DFP-sensitive form, and not by JHEH.

The role of JHEH in the regulation of the JH titre in *Drosophila melanogaster* is more important than in *D. virilis*. Evidently, it deals with the primary form of hormone, which could be the JHB_3 in *D. melanogaster* (Richard *et al.*, 1989; Moshitzky and Applebaum, 1995).

What may be the physiological implication of the high JH-hydrolytic activity in adults of *Drosophila*?

In their studies, Shapiro *et al.* (1986) have demonstrated that in adult females of the mosquito (*Aedes aegypti*), which is a representative of the *Diptera* order, eggs are cyclically oviposited and oviposition is associated with a sharp increase in the activity of JHE and, correspondingly, with a decrease in JH titre. The level of ecdysteroids concomitantly increases. The authors believe that the fall in JH titre is needed for termination of egg maturation and oviposition.

That a high level of JHE activity is requisite for normal maturation of eggs and for oviposition has also been concluded from studies of the enzyme in adults of the cabbage looper (*Trichoplusia ni, Lepidoptera*; Venkatesh *et al.*, 1988).

In *Drosophila*, in contrast to *A. aegypti*, maturation of eggs and oviposition is not cyclic; it is continuous. For this reason, there should exist eggs at the stage of vitellogenin accumulation needing high JH titre (Jowett and Postlethwait, 1981; Postlethwait and Parker, 1987; Bownes, 1989) and also eggs terminating maturation and ready to be oviposited, and this presumably calls for low level of hormone (Shapiro *et al.*, 1986; Venkatesh *et al.*, 1988).

We believe that maintenance of the required balance in the level of JH may be affected through intense synthesis of the hormone and its rapid degradation in those parts of ovaries where eggs are at the maturation stage. In this regard, the system of JH degradation acquires particular importance for reproduction in *Drosophila*.

The situation is presumably similar for the two species of *Lepidoptera*. In fact, Venkatesh *et al.* (1988) have shown that in the adult cabbage looper (*Trichoplusia ni*), the level of JHE activity is high, and this high level, as they believe, is necessary for normal reproduction and egg oviposition. On the other hand, in their laboratory it has been shown that in the tobacco hornworm (*Manduca sexta*) JHE activity rapidly falls after emergence, and it remains at a low level in the adult (Jesudason *et al.*, 1990), which contradicts the earlier suggested hypothesis of the role of JH-esterase in reproduction of *Lepidoptera*. The authors believe that these differences in the activity level of JHE between the adults of the cabbage looper and the tobacco hornworm reflect important physiological differences in the regulation of reproduction between these two species. When taking into account our data, it may be suggested that these differences between the cabbage looper and the tobacco hornworm are not related to crucial differences in their reproductive physiology, but rather to the fact that high JH-hydrolytic activity, needed for normal reproduction and oviposition, may be provided by different enzymes in these species, and this is what we observed for *D. virilis* and *D. melanogaster*. This has also been shown for two species of mosquitoes. While in females of *Aedes aegypty* the major JH-hydrolyzing enzyme is JHE (Shapiro *et al.*, 1986), and in *Culex quinquefasciatus* it is JHEH (Lassister *et al.*, 1994).

Support for the hypothesis that high activity of JHE is needed for reproduction of females of *D. virilis* may come from the fact that oviposition of fertilized eggs by females of line 101 starts on the next day after a maximum level of JHE activity is reached (see Fig. 3 and Table 2), i.e. 2 days after emergence, and females of line 147 become capable of ovipositing fertilized eggs on day 7 (see Table 2), i.e., here again, on the next day after a level of JH-esterase activity comparable with the one in individuals of line 101 is reached (see Fig. 3). The results of the experiments with injection of JHE also support the hypothesis that a precocious rise in JHE

activity elicits precocious onset of oviposition in line 147 (see Table 3).

As to the mutant lines 147 of *D. virilis* and 921283 of *D. melanogaster*, on the one hand a set of their feature is coincident, i.e. they are characterized by a lower level of JH degradation compared to wild type lines and, as shown earlier (Rauschenbach *et al.*, 1995; Gruntenko *et al.*, 1995), their JH metabolic system does not respond to stress. On the other hand, there are significant differences between the lines. In females of line 147 the activity level of the main JH-hydrolyzing enzyme, JHE, becomes "corrected" with age. There is no such correction in the activity level of the main JH-hydrolyzing enzyme, JHEH, in individuals of line 921283.

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