



# The Role of the Degradation System of the Juvenile Hormone in the Reproduction of *Drosophila* under Stress

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Two natural populations of *Drosophila melanogaster* were analyzed for the amount of the juvenile hormone (JH) degradation under normal and heat stress conditions. It was found that both populations are polymorphic for this character: the occurrence frequency of individuals with low level of JH hydrolysis, unaffected by heat stress, is high, being 56% and 64%, respectively.

The effect of heat stress on JH degradation and fertility was studied in *Drosophila virilis* and *D. melanogaster*. It was shown that the lines of wild type of both species respond to stressing effects by a decrease in the level of JH hydrolysis, delay in oviposition and also by a decrease in fertility for several days after exposure to the stress conditions. Experiments with inhibition of the juvenile hormone esterase (JHE) demonstrated that a decrease in the level of JH hydrolysis may be responsible for the response of the reproductive system of *Drosophila* to stress. It was shown that the in lines of *Drosophila* not responding to stressing agents by a decrease in the degradation level of JH, fertility is not altered under stress conditions. The role of JH in the reproduction of *Drosophila* under stress and in the adaptation of natural populations of *Drosophila* to stressing agents is discussed. Copyright © 1996 Elsevier Science Ltd

Juvenile hormone JH-esterase Epoxide hydrolase *Drosophila* Reproduction Heat stress

## INTRODUCTION

The stress response (Selye, 1972) as described for mammals is a problem of great interest to physiologists. Of particular interest is the study of the genetic control of the stress response, which has been evolutionary conserved and is characteristic of both mammals and insects (Rauschenbach *et al.*, 1987; Cymborowski, 1988; Ivanovic, 1991; Yankovic-Hladni, 1991). However, the mechanism operative in insects is not identical to the one established in mammals. The mechanism appears to involve a nonspecific neuroendocrine response, based on change in the relative proportions of hormones controlling development and function in insects (Cymborowski, 1991; Ivanovic, 1991; Rauschenbach,

1991; Rauschenbach *et al.*, 1993, 1995). Numerous studies have demonstrated that the JH and biogenic amines are important links in the stress response of insects (reviews; Cymborowski, 1991; Rauschenbach, 1991; Rauschenbach and Shumnaia, 1993).

The response of the metabolic systems of JH and biogenic amines to stress, as well as the genetic control of the response, have been under study for many years in this laboratory. Recently, we have demonstrated that young females (24h after adult emergence) of *D. melanogaster* and *D. virilis* responded to heat stress 38°C for 4h by a decrease in JH metabolism (Rauschenbach *et al.*, 1995). A mutant line, 147, has been identified among the studied lines of *D. virilis*. Mutant individuals of line 147 show a low level of JH metabolism, which is not affected by heat stress (Rauschenbach *et al.*, 1995). It was established that fertility of individuals of line 147 under normal conditions of reproduction is sharply decreased, and these individuals start to lay fertilized eggs only after their JH levels reaches that of wild type counterparts (Khlebodarova *et al.*, in press).

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Several questions arise because mutant line 147 was identified in wild populations: (1) How widespread are mutations of this kind in nature? (2) Why the mutations causing decrease in fertility under normal conditions can be retained in the populations? and (3) Could the presence of such mutations in wild populations be related to the role JH metabolic systems play in reproduction of *Drosophila* under stress conditions?

Attempts to answer these questions will be made in the present study.

## MATERIAL AND METHOD

To characterize populations, we analyzed a set of isofemale lines of *D. melanogaster* obtained from females which were mated in nature and caught from two populations of the Altai territory, Gorno-Altai and the Pospelikha settlement.

The 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* in chromosome II and temperature-sensitive lethal in chromosome VI. The two lines of *D. virilis* are contrasting 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 Gorno-Altai by individual crosses through five successive generations; they also differ in their response to stress (Gruntenko *et al.*, in press). Because these differences in JH metabolism and response to stress were established only for adult females of *Drosophila* (Rauschenbach *et al.*, 1995), our subsequent studies, including the present, are concerned with females only.

Cultures of both species were grown on standard nutritive medium (Rauschenbach *et al.*, 1987) at 25°C at the density of 20 larvae/7 ml of the medium. Cultures of both species were synchronized by hatching and fly emergence. Individuals of both species, controls and those exposed to heat stress, were frozen in liquid nitrogen and stored at -20°C until further use. The experiments were performed in winter.

Measurement of hydrolysis of radioactive JH were carried out by the method of (Hammock and Sparks, 1977). A fly was homogenized on ice in 30  $\mu$ l of 0.1 M Naphosphate 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. 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  $\mu$ g unlabeled JH-III (Sigma) and 12,500 dpm 3H chain labelled 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  $\mu$ l of incubation mixture at 37°C.

The products of hydrolysis of labeled JH-III were ana-

lyzed by TLC according to Renucci (1986). The reaction products were extracted with ethyl acetate and applied to silica gel plates (60 F 254 'Merck'). To clearly separate JH-acid-diol and JH-diol, chromatography was performed as follows: chromatography was carried out in a toluene:ethyl acetate medium (1:1, V/V) to a height of 3.5 cm; the plate was dried, and chromatography was continued in a toluene:ethyl acetate:acetic acid medium (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 toluene scintillator, and the amount of radioactivity was measured in a Delta-300 counter.

Experiments for determination of fertility of *D. virilis* and *D. melanogaster* were designed as follows. Freshly emerged females and males of *Drosophila* were placed into vials with food (five females and five males per vial, 15–20 vials into each control and experimental groups). The flies were placed on fresh food daily, the procedure was repeated for 8–12 days. All the vials with laid eggs were left undisturbed until fly emergence. In the experiments with heat shock females were separated from males 16h before the experiment was started (both in the experimental and control series) and males were returned to appropriate vials after stress. This was necessary because, as preliminary experiments established, males of *Drosophila* become sterile after heat stress of such long duration.

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

## RESULTS

### *Analysis of natural populations of D. melanogaster for level of JH hydrolysis under normal conditions and heat stress*

Before analyzing the level of JH hydrolysis in individuals of *D. melanogaster* of natural populations, we analyzed for the parameter laboratory stock of Canton-S. The distribution of females of this stock according to JH hydrolysis level under normal conditions and heat stress is shown in Fig. 1 (left and right histograms,

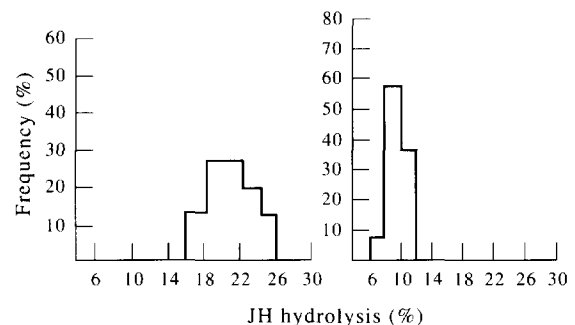


FIGURE 1. JH metabolism in Canton-S females of *D. melanogaster* under normal (left histogram) and heat stress (right histogram) conditions.

respectively). It is clearly seen that Canton-S is homogeneous both with respect to JH hydrolysis level and the capacity to respond to stress: there is a single class in the distribution under normal conditions; these are individuals with high JH hydrolysis level ranging from 16–26%, and a decrease to 6–12% of JH hydrolysis is observed in all the females of these lines after a 3h exposure to 38°C.

To characterize *D. melanogaster* of the Pospelikha population, 31 isofemale lines were analyzed (1–2 flies of each line) under normal conditions and heat stress. Figure 2 presents the distribution of the level of JH hydrolysis in females of this population. Clearly, under normal conditions (left histogram) two distinct classes are distinguished in the histogram: one with a low level of JH hydrolysis from 8–16% (at a frequency of 55.8%), the other with a high one ranging from 18–44% (at a frequency of 44.2%). The distribution obtained, when JH hydrolysis was measured after exposure of flies of the given population for 3h at 38°C (right histogram) is virtually represented by a single class with a peak corresponding to the class with the low level of JH hydrolysis in control flies. This distribution seems to indicate that, in individuals of the class with a high level of JH hydrolysis, the latter decreases under stress conditions. It should be noted that, in individuals of the class with a low level of hydrolysis of hormone, JH metabolism under stress obviously does not alter.

To characterize *D. melanogaster* of the Gorno-Altai population, 12 isofemale lines were examined, 3–6 flies of each line. The distribution of the level of JH hydrolysis in females under normal conditions (left histogram) and after 3h exposure at 38°C (right histogram) is shown in Fig. 3. Under normal conditions the same classes are present with similar frequency (64% and 36%, respectively) in Gorno-Altai population as in the Pospelikha population. The distribution of this population under stress is also represented by a single class. Thus, mutant individuals with a low level of JH hydrolysis resistant to change under the effects of unfavourable conditions are widespread in natural populations of *D. melanogaster*.

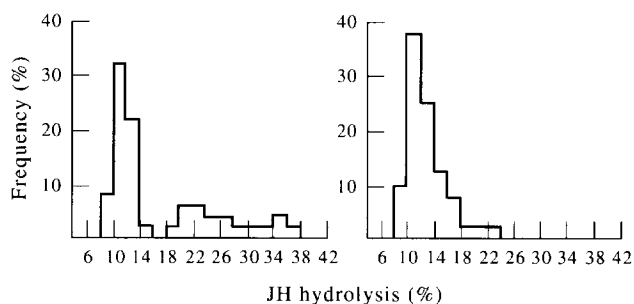


FIGURE 2. JH metabolism in females of *D. melanogaster* from Pospelikha population under normal (left histogram) and heat stress (right histogram) conditions.

#### *Activity of enzymes metabolizing JH during reproduction of D. virilis and D. melanogaster under normal conditions and heat stress*

To elucidate the role of JHE and epoxide hydrolase of the juvenile hormone (JHEH), which are the key enzymes in JH degradation, in reproduction of *Drosophila* under stress, we studied the activity of these enzymes under normal conditions and brief heat stress in 1-day and 5-day old females of *D. virilis* and 1-day and 5-day old females of *D. melanogaster*. The results are set out in Fig. 4.

As shown from the data of Fig. 4(A) the activity of JHEH in *D. virilis* changes neither with age, nor under heat stress. The enzyme activity is the same in individuals of wild type (line 101) and in those of mutant line (147).

The activity of JHE is appreciably lower in young mutant individuals compared to flies of the wild type, and JHE activity levels off with age. As we have previously shown (Khlebodarova *et al.*, in press), thereafter flies of line 147 acquire the capacity to lay eggs.

In mutant flies (line 147) of both age groups, JHE activity does not change under stress. Decrease in JH hydrolysis in flies of line 101 of both age groups is due to lowering of JHE activity [Fig. 4(A)].

As in *D. virilis*, the activity of JHE and JHEH do not change significantly under the stress either in young or 4-day old females [Fig. 4(B)] in the mutant line of *D. melanogaster* which is unresponsive to stress (921283). However, in contrast to *D. virilis*, the decrease in JH hydrolysis under stress is influenced through a decrease in the activities of both JHE and JHEH in young females of wild type (Canton-S) and through a decrease in the activity of only JHEH in 4-day old females [Fig. 4(B)].

#### *Characterization of reproduction of Drosophila species under normal conditions and heat stress*

Bearing in mind the role of JH in the control of insect reproduction, we studied the fertility of wild type flies (line 101 of *D. virilis* and Canton-S of *D. melanogaster*) and that of mutant (line 147 of *D. virilis*) under normal conditions and after heat stress. To reiterate, only females were exposed to heat stress.

The results of the study of the effect of heat stress on the fertility of mated females of line 101 of *D. virilis* are summarized in Fig. 5.

Heat stress produces a one day delay in oviposition in females of line 101 that had just started laying eggs and decreases the fertility during the next five days [Fig. 5(A)]. Thereafter fertility increases, reaching the control level 8 days after stress. In females of line 101, 6 days after the beginning of oviposition, stress brings oviposition to a halt for 2 days and causes a more considerable decrease in fertility compared with females that had just started to lay eggs [Fig. 5(B)].

The activities of JHE and JHEH in stressed mated females (line 101) 6 days after the beginning of oviposition are shown in Fig. 5(C). As follows from the data

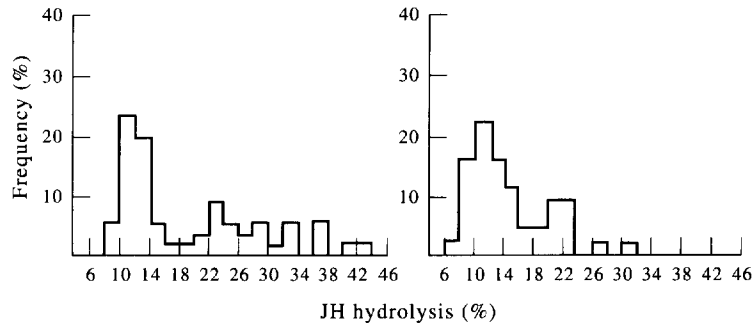


FIGURE 3. JH metabolism in females of *D. melanogaster* from Gorno-Altai population under normal (left histogram) and heat stress (right histogram) conditions.

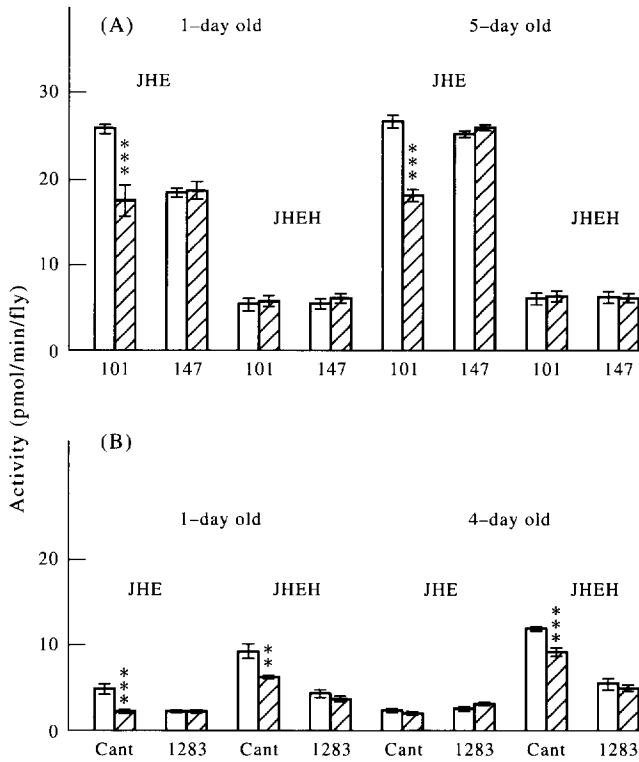


FIGURE 4. Effect of heat stress on JHE and JHEH activities in 1- and 5-day-old females of *D. virilis* (A) and in 1- and 4-day-old females of *D. melanogaster* (B). 101 and 147 – lines 101 and 147 of *D. virilis*, Cant and 1283 – lines Canton-S and 921283 of *D. melanogaster*, clear columns – Controls, shaded columns – heat-stressed.

of this figure, JHE activity in the treated females 24h after stress is still considerably lower in the heat-treated females than controls (the difference is significant,  $P < 0.01$ ), and it is persistently lower than in controls, even after 48h (the difference is significant,  $P < 0.05$ ).

Figure 6(A) presents the data on the effect of heat stress on egg laying by virgins of line 101 of *D. virilis*. A day after the onset of oviposition stress produces in virgins, just as in mated females, a cessation of egg laying lasting for a day. The activity of JHE in such females sharply follows [Fig. 6(B)]. Four days after stress, the heat-stressed virgins start to lay eggs in the same number as control flies [Fig. 6(A)].

The results of the experiments designed to study the

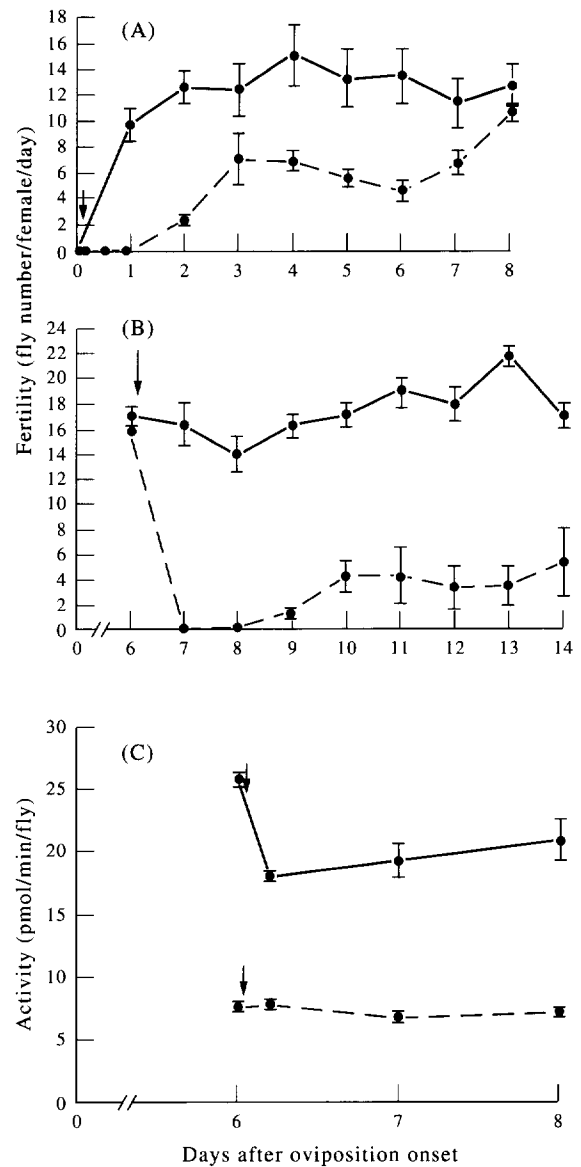


FIGURE 5. Effect of heat stress on fertility and on JHE and JHEH activities of 101 line mated females of *D. virilis*. A – stress on the first day after the oviposition onset; B – stress through 6 days after the oviposition onset, continuous line – control, discontinuous line – heat-stressed; C – stress through 6 days after the oviposition onset, continuous line – JHE, discontinuous line – JHEH.

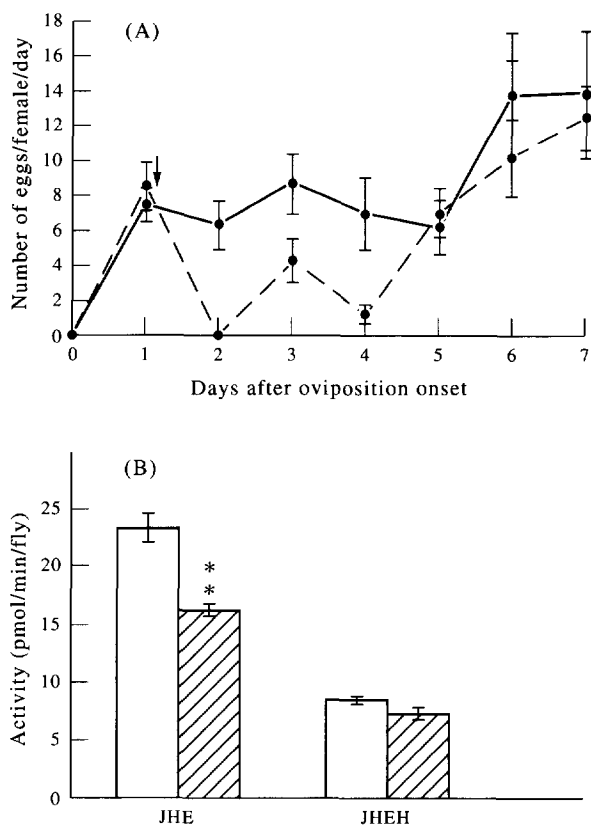


FIGURE 6. Effect of heat stress on oviposition (A) and on JHE and JHEH activities (B) of 101 line virgins of *D. virilis*. Continuous line – control, discontinuous line – heat-stressed on the first day after the oviposition onset.

effect of heat stress on the fertility of Canton-S stock of *D. melanogaster* are shown in Fig. 7. A day after emergence, stress produces in mated females of *D. melanogaster*, like those of *D. virilis*, a delay of one day in oviposition and then a decrease in fertility. However, it takes less time for fertility to be restored in *D. melanogaster*, than in *D. virilis* (Fig. 7). Similarly to *D. virilis*, the effect of stress on intensely reproducing females of *D. melanogaster* (stress exposure after 3 days after fly emergence) proved to be more marked: oviposition

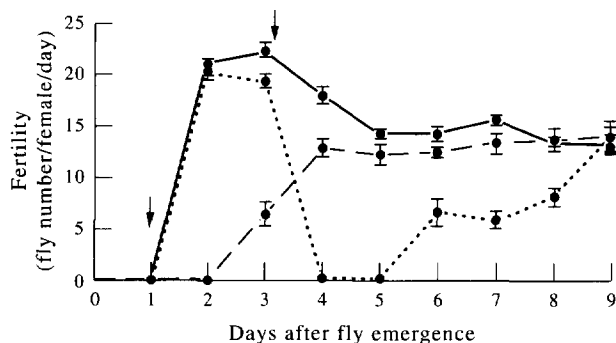


FIGURE 7. Effect of heat stress on fertility of Canton-S line mated females of *D. melanogaster*. Continuous line – control, discontinuous line – heat-stressed through 1 day after emergence, dotted line – heat-stressed through 3 days after emergence.

ceased for 2 days and the subsequent decrease in fertility was more protracted. The control level of fertility was reached only 5 days after stress.

Figure 8 presents data on the effect of heat stress on fertility of females of mutant line 147 of *D. virilis*. As we have shown (Khlebodarova *et al.*, in press), flies of line 147 differ in lower fertility from the wild under normal conditions. And, as seen in Fig. 8, stress is without effect on the fertility of females of this line. This is in good agreement with the observation that stress does not affect the activity of enzymes degrading JH in females of line 147 [see Fig. 4(A)].

#### *The role of JHE on the reproduction of Drosophila species under stress conditions*

To clarify the role of JHE in the delay in oviposition after stress exposure, we studied the effect of application of the specific inhibitor of JHE in *Drosophila* (Rauschenbach *et al.*, 1995) 3'-octyl-thio-1, 1-trifluoropropanone (OTFP) on the reproduction pattern in wild type lines 101 of *D. virilis* and Canton-S of *D. melanogaster*. Female fertility was studied after applying 0.5  $\mu$ l OTFP diluted in ethanol at a concentration of 8 mM. An application of 0.5  $\mu$ l of ethanol was used for control individuals.

A single application of OTFP produces a delay in oviposition for 20h in females of line 101 of *D. virilis*, then an increase in fertility for the next day (Fig. 9). Seventy-two hours later, OTFP ceases affecting female fertility. Proceeding from the data of Roe *et al.* (1993) according to which OTFP degrades rapidly *in vivo*, we performed experiments where OTFP was applied topically two times. OTFP was applied for the second time 7h after the first. The results of this experiment are also shown in Fig. 9. The delay in oviposition in this experiment was longer (at least 24h), and fertility was decreased during the subsequent two days. Seventy-two hours after the double application of OTFP, fertility returned to the control level.

The activities of JHE and JHEH in females of line 101, in the case of OTFP application, are given in Table 1.

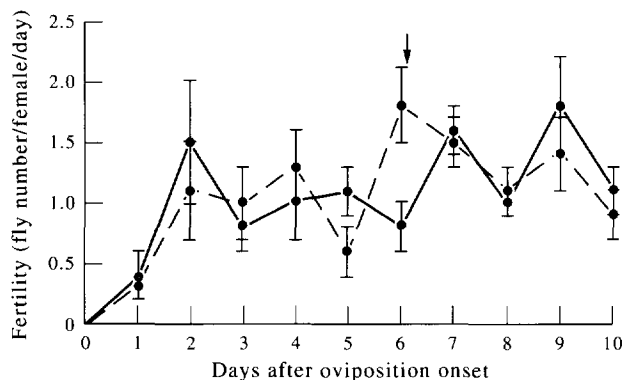


FIGURE 8. Effect of heat stress on fertility of 147 line mated females of *D. virilis*. Continuous line – control, discontinuous line – heat-stressed through 6 days after the oviposition onset.

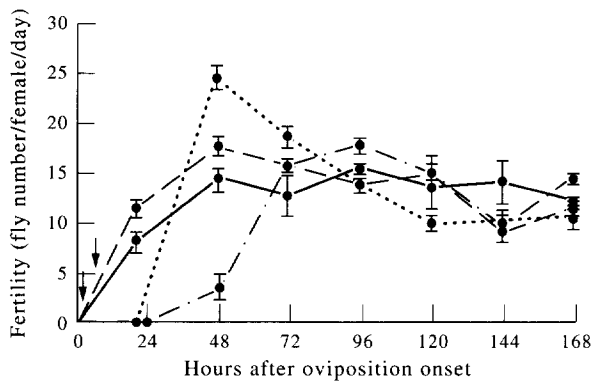


FIGURE 9. Effect OTFP on fertility of 101 line mated females of *D. virilis* (OTFP application on the first day after the oviposition onset). Continuous line – intact control, discontinuous line – ethanol application, dotted line – single OTFP application, discontinuous-dotted line – double OTFP application.

TABLE 1. Effect of OTFP on JHE and JHEH activities in mated females of 101 line of *D. virilis*

Group	Number of flies	Activity (pmol/min/fly) $\pm$ SE	
		JHE	JHEH
Control	12	24.1 $\pm$ 0.9	6.6 $\pm$ 0.5
Experiment: through 4h after first application	8	15.6 $\pm$ 1.3***	6.8 $\pm$ 0.3
through 7h after first application	6	19.5 $\pm$ 1.8*	7.1 $\pm$ 0.4
through 16h after second application	6	16.4 $\pm$ 0.8***	6.8 $\pm$ 0.6

\*\*\*The difference from control is significant,  $P < 0.001$

\*The difference from control is significant,  $P < 0.05$ .

From the data in the table the inhibitory activity is transient and, it may be inferred that OTFP, indeed, degrades quite rapidly *in vivo*. In fact, the activity of JHE starts to rise 7h after its application. However, the second application of OTFP maintains JHE activity at a low level for an appreciably longer time period than a single application. This is probably the reason why fly fertility is lower during the several days following the double application of OTFP.

The results of experiments with a single application of OTFP to females of Canton-S line of *D. melanogaster* are shown in Fig. 10. Topical application of OTFP to females of Canton-S line does not elicit a delay in oviposition, it induces, however, an increase in fertility two days after application, like in *D. virilis*. OTFP ceases to affect fertility of females of *D. melanogaster* 3 days after application.

## DISCUSSION

It is known that JH controls the growth of previtellogenic follicles and the accumulation of vitellogenins pass-

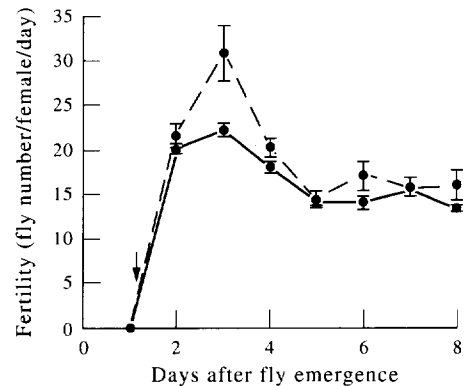


FIGURE 10. Effect OTFP on fertility of Canton-S mated females of *D. melanogaster* (OTFP application on the first day after the oviposition onset). Continuous line – control (ethanol application), discontinuous line – OTFP application.

ing from the haemolymph to oocytes. JH must be applied at a high level to initiate maturation of the ovaries and stimulate vitellogenesis, and then at lower levels to maintain vitellogenesis and the production of vitellogenins by fat body (Jowett and Postlethwait, 1981; Raikhel and Lea, 1985; Postlethwait and Parker, 1987; Adams *et al.*, 1988; Bownes, 1989). There are data suggesting that for completion of normal egg development and oviposition, the JH titre must be reduced (Shapiro *et al.*, 1986). This reduction is thought to be effected by JHE in some species and by JHEH in others (Shapiro *et al.*, 1986; Lassister *et al.*, 1994; Khlebodarova *et al.*, in press). We have previously suggested that maintenance of a balance in JH levels required for the regulation of growth, maturation and laying of eggs in *Drosophila* may be effected through intense synthesis of hormone and its rapid degradation in those parts of the ovaries where eggs are approaching maturity (Khlebodarova *et al.*, in press). From other previous data it also follows that this degradation is accomplished through JHE in *D. virilis* and mainly through JHEH in *D. melanogaster* (Khlebodarova *et al.*, in press). This conclusion is also supported by analysis of the activities of enzymes metabolizing JH, as the results presented here demonstrate (see Fig. 4). Interspecific differences of this kind have been also established for mosquitoes: JHEH is the predominant route for JH metabolism in *Culex quinquefasciatus* (Lassister *et al.*, 1994), while the JHE route predominates for it in *Aedes aegypti* (Shapiro *et al.*, 1986).

Stress exposure elicits in females of *Drosophila* a decrease in the activities of enzymes degrading JH: it is JHE in *D. virilis*, JHEH and JHE in young individuals of *D. melanogaster* and JHEH in intensely reproducing females of *D. melanogaster* (see Fig. 4). If a decrease in JH titre is required to provide oviposition in the given species, as we suggested (Khlebodarova *et al.*, in press), heat stress, by inducing a decrease in the activities of enzymes hydrolyzing JH (and, as a consequence, presumably, a rise in JH level) should lead to a delay of oviposition. The results summarized in Figs 5 and 7 support

this assumption. Indeed, stress exposure elicits in young females of both *Drosophila* species, a 24h delay of oviposition onset with a subsequent decline in the fertility for some days. As for the intensely reproducing females, oviposition ceases for two days.

A response of this kind is also a characteristic feature of virgins of *D. virilis*: stress elicits a sharp fall in the activity of JHE and also halts oviposition for a day with a subsequent decline in the number of eggs laid for 3 days (see Fig. 6). A decline in the fertility owing to heat stress in *D. melanogaster* was also demonstrated by Krebs and Loeschke (1994). However, the authors estimated the fertility by transferring the flies to fresh food vials once in three days and that is why they cannot detect a delay in oviposition onset after stress exposure.

It should be noted that, according to our data, JHE activity is at the same level in virgins and mated females of *D. virilis*. It should be stipulated that (Venkatesh *et al.*, 1988) have demonstrated a lower level of JHE activity for mated females compared with virgins of *Trichoplusia ni* (Lepidoptera). This difference may be due to the different reproductive physiology of Diptera and Lepidoptera. Another possible explanation is that Venkatesh *et al.* (1988) have measured JHE activity in the haemolymph, while we have measured it in the whole fly. Thus, in experiments with *T. ni*, only JHE activity was taken into account, which decrease in mated females is required to maintain a high JH titre in the haemolymph to provide the production of vitellogenins by fat body and their absorption from the haemolymph by the oocytes. The decrease in JH titre required for completion of egg maturation and oviposition may be local, and it may be effected through JHE and/or JHEH only in those parts of the ovary, where eggs are at the last stages of maturation. This JH-hydrolyzing activity was disregarded in the experiments of Venkatesh *et al.* (1988).

As known, stress produces substantial changes in hormonal balance and total metabolism in insects (see review: Cymborowski, 1991; Jankovic-Hladni, 1991; Rauschenbach, 1991). With this in mind, it could not be excluded that a delay in oviposition in *Drosophila* may not be influenced by JH metabolism.

To clarify this issue, we studied the effect of application of OTFP, a selective JHE inhibitor in *Drosophila* (Rauschenbach *et al.*, 1995), on the reproduction pattern of wild-type lines of *D. virilis* and *D. melanogaster*. A single topical application of OTFP in *D. virilis*, like heat stress, produced a delay in oviposition (see Fig. 9), thereby supporting our hypothesis that the JH degradation system controls delayed oviposition under stress in this species. The revealed dose-dependence (double application OTFP), increasing the time of JHE inactivation, prolongs the delay in oviposition and produces a decrease in fertility (see Table 1 and Fig. 9), also tends to support this idea.

Absence of delay in oviposition in the case of OTFP application in *D. melanogaster* (see Fig. 10) support the hypothesis that the effect observed in *D. virilis* is spec-

ific, i.e. inhibition of the enzyme hydrolyzing JH causes delay in egg laying in this species. In fact, as indicated above, JHEH is the predominant route of JH metabolism in adults of *D. melanogaster*, and OTFP is without effect on the activity of this enzyme in this species (Rauschenbach *et al.*, 1995) and therefore, OTFP can not produce a considerable decrease in JH hydrolysis in *D. melanogaster*.

We have thus established that exposure of adults of *Drosophila* to extreme environmental conditions causes a decline in activities of enzymes degrading JH in females and, possibly, a rise in JH level. A hormonal response of this kind to stress in adults produces a delay in oviposition, which, in all probability, promotes adaptation to strong stress, allowing the population to survive until hard times are over.

However, monitoring natural population of *D. melanogaster*, disclosed that they are polymorphic for the metabolic level of JH and stress response (see Figs 2 and 3). In both populations with a high frequency, there occur individuals with a low level of hydrolysis of hormone, not responding to stress by its decrease.

Because both *D. melanogaster* populations were taken from areas under impact of man-made stressors, we assumed that the situation may reflect existence of *Drosophila* population under conditions of frequent stressing exposures of low intensities. Under such conditions, stress-responsive individuals respond to noxious agents by delayed oviposition (see Figs 5 and 7); stress-unresponsive individuals keep reproducing at their usually low fertility level (see Fig. 8). Hence, under conditions of frequent exposure to stress of low intensity, the latter have imparted an advantage in the production of viable offspring and this most likely leads to an increase in the number of such advantageous individuals in the population.

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