5 Gammon, D.W., Brown, M.A., and Casida, J.E., Pestic. Biochem. Physiol. *15* (1981) 181.
6 Scott, J.G., and Matsumura, F., Pestic. Biochem. Physiol. *19* (1983) 141.
7 Lawrence, L.J., and Casida, J.E., Science *221* (1983) 1399.
8 Georghiou, G.P., Exp. Parasit. *26* (1969) 224.
9 Tsukamoto, M., Botyu-Kagaku *29* (1964) 51.
10 Yates, F., in: The Design and Analysis of Factorial Experiments. Harpenden, 1937.
11 Sawicki, R.M., Nature *275* (1978) 443.
12 Hoyer, R.F., and Plapp, F.W. Jr, J. econ. Ent. *59* (1966) 495.
13 DeVries, D.H., and Georghiou, G.P., Pestic. Biochem. Physiol. *15* (1981) 234.
14 Tsukamoto, M., in: Pest Resistance to Pesticides, p.71. Eds G.P. Georghiou and T. Saito. Plenum, New York 1983.
15 Milani, R., 11th Int. Congr. Ent. *3* (1960) 232.
16 Georghiou, G.P., Proc. 2nd Int. Congr. Pestic. Chem. *2* (1971) 77.
17 Hoyer, R.F., and Plapp, F.W. Jr, J. econ. Ent. *61* (1968) 1269.
18 Sawicki, R.M., Pestic. Sci. *4* (1973) 171.
19 Tate, L.G., and Plapp, F.W. Jr, Biochem. Genet. *11* (1974) 49.
20 Kahn, M.A.Q., Morimoto, R.I., Bederka, J.P. Jr, and Runnels, J.M., Biochem. Genet. *10* (1973) 243.

0014-4754/84/121416-03$1.50 + 0.20/0

Sexual behavior without adult morphogenesis in *Locusta migratoria*

U. Shalom and M.P. Pener[1]

Department of Zoology, The Hebrew University, Jerusalem (Israel), 5 January 1984

Summary. Injection of azadirachtin into young Vth (= last) instar *Locusta* hoppers inhibits molt and a considerable portion of the animals survive for more than 40 days as 'over-aged nymphs'; in contrast, the duration of the Vth instar is 8–10 days in normal controls. Males of over-aged nymphs exhibit adult sexual behavior, and injection of juvenile hormone intensifies this behavior. The results demonstrate that the 'terminal' molt leading to morphogenetic adult differentiation is not necessary for an adult behavioral pattern to develop, and/or to become overt, in a hemimetabolous insect.
Key words. Azadirachtin; sexual behavior; ontogenetic relations between morphology and behavior; juvenile hormone; *Locusta migratoria.*

Azadirachtin, extracted from neem seeds[2–4], acts as an anti-feedant and/or a growth regulator in many insects. It inhibits feeding, disrupts growth, delays or inhibits molt, interferes with completion of the molt and/or induces morphogenetic disturbances in various species[2, 5–12], and often causes high mortality. It also reduces ecdysteroid titre in the hemolymph[12–14]. We injected azadirachtin into Vth (= last) instar *Locusta* hoppers, in order to inhibit the molt and to obtain 'over-aged' nymphs. Then we assessed the male mating behavior of the over-aged nymphs in order to investigate whether the ontogenetic development of an adult behavioral pattern depends on overt morphogenetic adult differentiation, and to what extent adult behavior is correlated with adult morphology.

Crowded nymphs of *Locusta migratoria migratorioides*, from the Jerusalem stock culture[15], were kept under previously described conditions[16]. Azadirachtin, kindly supplied by Prof. H. Rembold, was injected in 2 µl ethanol 90%, through the lateral side of the abdomen, into Vth (= last) instar nymphs, 2–8 h after the molt to this instar.

Following injection of 1.6 µg azadirachtin per nymph, 2 major kinds of mortality were observed (table). A portion of the nymphs showed markedly delayed apolysis and actually started to molt, but were unable to shed the exuvia and died in the course of this unsuccessful molt. Such 'death in molt' became less frequent after the age of 30 days (here and below the age refers to the time elapsed after the molt to the Vth instar) and did not occur after 40 days. Another portion of the animals died as Vth instar nymphs, either without apolysis, or after apolysis but without attempting to molt. This kind of mortality, termed 'death not in molt', was common up to the age of 20 days, but became less frequent later, though it occurred up to 50 days and even beyond this age (see footnotes to table). In spite of all the mortality, about 25% (table, exp. 1) or 48% (table, exp. 2) of the locusts survived more than 20 days as over-aged Vth instar nymphs and respectively 16% and 20% of them survived more than 40 days in this state; the duration of the Vth instar was 8–10 days in the controls. The last azadirachtin-injected nymphs, out of those observed for their whole life span, died at the age of 69 and 71 days, not in molt. This age approaches the life span of normal adults[17, 18]. Very recently Sieber and Rembold[12] described the lethal and molt delaying/inhibiting effects of azadirachtin injected into IVth and Vth instar *Locusta* hoppers. Their presentation of the results is different from that employed by us in the table (our aim was to obtain a maximum number of over-aged nymphs for behavioral studies), but insofar as the findings are comparable, they agree well. The dose of 1.6 µg azadirachtin was optimal for obtaining the highest proportion of over-aged Vth instar nymphs. With lower doses of 1.2 and 0.8 µg azadirachtin, the proportion of nymphs showing death in the molt markedly increased, and with 0.4 µg about 45% of the nymphs completed the moult to normal adults. Higher doses, 3.2 and

Mortality and effect on delay/inhibition of the molt caused by injection of 1.6 µg azadirachtin (in 2 µl ethanol 90%) to 2–8-h-old Vth instar *Locusta* nymphs*. In parenthesis: % of No. treated

Experiment	No. treated	No. died in molt within the age of			No. died not in molt within the age of				No. survived as Vth instar nymph over 40 days
		9–20 days	21–30 days	31–40 days	1–10 days	11–20 days	21–30 days	31–40 days	
1	24	1 (4.2)	2 (8.3)	0 (0.0)	14 (58.3)	3 (12.5)	0 (0.0)	0 (0.0)	4** (16.7)
2	115	8 (7.0)	22 (19.1)	2 (1.7)	24 (20.9)	27 (23.5)	3 (2.6)	6 (5.2)	23*** (20.0)

* Out of 24 controls injected with 2 µl ethanol, 23 molted to normal adults within the age of 8–10 days and 1 died at the age of 3 days; ** 1 died at the age of 42 days, 2 at the age of 69 days and the last one at the age of 71 days; none of these deaths occurred in the molt; *** 9 died between the age of 41–48 days, none of them in the molt. The remaining 14 were not followed over 48 days.

6.4 µg azadirachtin, caused 79% and 100% mortality respectively, not in molt, within the age of 1–10 days.

Vth instar nymphs were injected with 1.6 µg azadirachtin as above. At the beginning of the 3rd week the surviving male nymphs were marked individually and observations on their mating behavior (see below) were initiated. On day 21 (beginning of the 4th week) the over-aged male nymphs were divided into 2 groups. The males of 1 group were injected on day 21 with 144 µg JHIII (juvenile hormone III, Fluka Chem. Comp.) in 2 µl olive oil and on the next day with the same dose again. The males of the other group and all females received no injection. Observations on male sexual behavior were continued during the 4th, 5th and 6th weeks. Mortality (of either kind) did not stop during this period, but it did not differ in the 2 groups.

After the observations on sexual behavior had begun the males were kept in 12-l cages, 3–10 males per cage, together with 4–7 over-aged Vth instar females. For the period of actual observations 3–4 normal adult females were added to each cage. These observations were carried out 3–5 times per week, for 120 min each time. At 10-min intervals the position of each male was noted; mounting a female (or another male), bending of the abdomen towards the female's genitalia, etc., were considered as male mating behavior. The weekly average percentage of time spent on sexual behavior was calculated for each group of males (see Wajc and Pener[17] for details). The higher this percentage, the more intense was the sexual behavior.

From the qualitative standpoint, male sexual behavior (mounting, trembling, bending of the abdomen, searching with the tip of the abdomen for the female's genitalia) shown by the over-aged nymphs (fig. 1) did not differ markedly from that exhibited by normal adult males, except that the over-aged nymphs were unable actually to copulate. Their external genitalia were those of Vth instar nymphs, and still useless for copulation. Over-aged male nymphs exhibited sexual behavior toward both over-aged female nymphs and normal adult females.

A few over-aged male nymphs started to show some sexual behavior already during the 3rd week (days 14–20); the intensity of their mating behavior increased during the 4th week and reached a maximum, 20–25% of time spent on sexual behavior, in the 5th and 6th weeks (fig. 2). The intensity of male sexual behavior increased more markedly in JH-injected over-aged nymphs and it reached a maximum, 45%, during the 5th week (7–13 days after first JH injection), but then declined considerably (fig. 2). For comparison, normal crowded adult males reach full sexual maturation in the 3rd or 4th week of their adult life and show weekly averages of 60–80% of time spent on sexual behavior[17,19]. So, the over-aged nymphs, even those injected with JH, exhibited less intense male mating behavior than normal adults and they reached maximum intensity about 1.5 week later than adults. This delay corresponds well to the duration of the Vth instar in normal locusts (8–10 days). Thus, counting from the molt to the Vth instar, normal adult males and Vth instar over-aged male nymphs reach maximum sexual behavior at the same age!

The individual marking allowed us to observe that all over-aged male nymphs surviving up to the end of the 5th week showed at least some sexual behavior at some time, though one (only azadirachtin-treated) male nymph first exhibited it as late as the beginning of the 6th week. These findings show that if the nymphs survive sufficiently long, the behavioral pattern of adult sexual behavior eventually does develop and/or becomes overt in all of them. Moreover, male sexual behavior was exhibited also by those over-aged nymphs which eventually died in molt. Sexual behavior, therefore, is not prevented even when the over-aged nymphs actually reach the terminal molt, though the detailed data indicate that this behavior may not be exhibited within the last days before actual death in molt, possibly because during these last days locomotor activity is substantially reduced.

We did not attempt to investigate the mode of action of the azadirachtin in inhibiting molting, and used the substance only as a tool for obtaining over-aged Vth instar nymphs. The results, however, strongly indicate that azadirachtin does not act solely on food intake or digestion. It is highly improbable that interference just with feeding or food utilization would delay molt up to the age of 40 days and would result in the survival

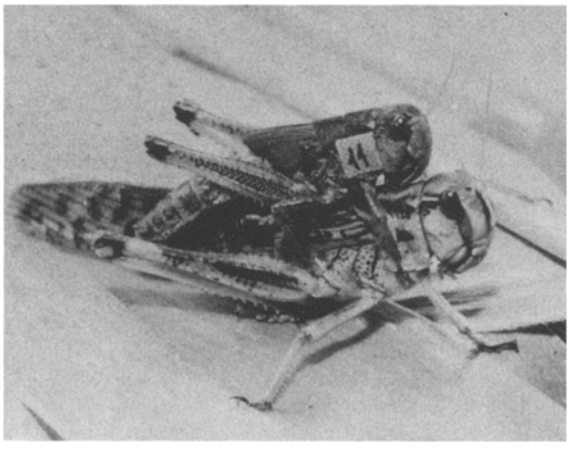

Figure 1. Over-aged Vth instar male nymph of *Locusta* (obtained by injection of azadirachtin at the beginning of the instar) exhibiting sexual behavior toward a normal adult female. Note bending of the male's abdomen, characteristic of sexual behavior, in search of the female's genitalia.

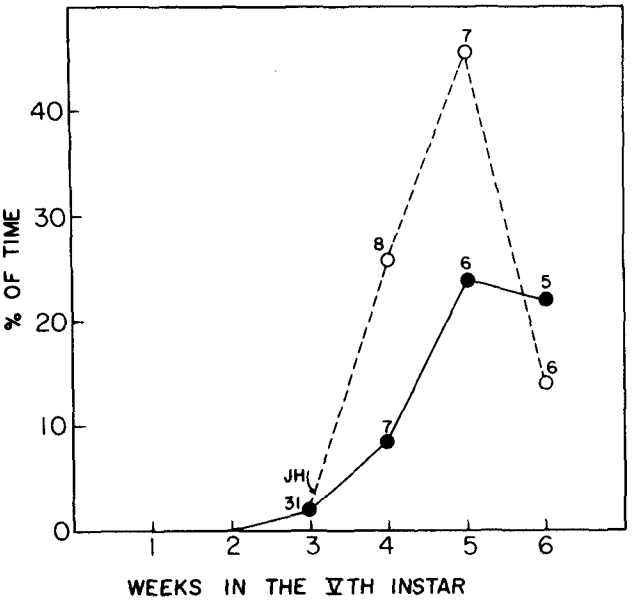

Figure 2. The intensity of sexual behavior exhibited by over-aged Vth instar male nymphs of *Locusta*, expressed in average percentage of time spent on sexual behavior[17,19], during the consecutive weeks of the experiments. Data are based on 3–5 observations per week, 2 h each time. Numbers close to signs show number of over-aged male nymphs surviving till the end of the given week (= minimum number of nymphs on which data are based for the same week). Dots and continuous line: nymphs injected with 1.6 µg azadirachtin in 2 µl ethanol 90%, 2–8 h after the molt to the Vth instar. Circles and scattered line: nymphs treated as above but injected on day 21 (beginning of the 4th week) with 144 µg JHIII (juvenile hormone III) in 2 µl olive oil and on day 22 with the same dose again; sign JH with arrow shows the timing of these JH-injections.

of some other over-aged Vth instar nymphs, without molt, up to 71 days. Azadirachtin, therefore, may have anti-hormonal effects[9,11-13], at least in addition to its anti-feedant action.

Precociously metamorphosed adultiforms of *Locusta*, obtained by surgical allatectomy or by precocene-treatment of younger instars, do exhibit male sexual behavior[19-20]. Thus, metamorphosis promoting endocrine events, or experimentally induced prothetely, exert a major effect on this behavior, allowing it to become overt, or even enhancing its ontogenetic development[19]. But adultiforms have a terminal molt, which though it does not lead to morphologically normal adult, nevertheless results in a considerable morphogenetic adult differentiation[16]. Thus, although endocrine interferences enhancing prothetelic metamorphosis do induce earlier appearance of male sexual behavior[19] (and also of some other adult behavioral patterns[21,22]), the present results show that the reverse of this conclusion is not true; delay or inhibition of the terminal molt neither prevents, nor delays, the appearance of such behavior. Moreover, exogenous JH intensifies male sexual behavior of crowded over-aged Vth instar nymphs, as does endogenous or exogenous JH in normal crowded adults[17,19,23].

Several questions remain open. It is unclear why male sexual behavior exhibited by over-aged Vth instar nymphs is less intense than that of normal adults. We know nothing about the functioning of the CA and JH metabolism in azadirachtin-treated over-aged nymphs; intensification of their male mating behavior by exogenous JH may as well reflect a low endogenous JH titre as a necessity for extra JH due to a possible lower response of the system underlying this behavior to the hormone. It is clear, however, that overt morphogenetic adult differentiation is not necessary for male sexual behavior to develop and/or to become overt in a hemimetabolous insect. Thus, overt adult behavior and overt adult morphology seem to be independent to a considerable extent in this case.

1 We thank Prof. H. Rembold for a generous gift of azadirachtin and Mr Y. Toren for keeping the locust culture.

2 Butterworth, J.H., and Morgan, E.D., J. Insect Physiol. *17* (1971) 969.
3 Zanno, P.R., Miura, I., Nakanishi, K., and Elder, D.L., J. Am. chem. Soc. *97* (1975) 1975.
4 Nakanishi, K., Rec. Adv. Phytochem. *9* (1975) 283.
5 Gill, J.S., and Lewis, C.T., Nature *232* (1971) 402.
6 Ruscoe, C.N.E., Nature New Biol. *236* (1972) 159.
7 Warthen, J.D. Jr, Redfern, R.E., Uebel, E.C., and Mills, G.D. Jr, USDA, Agric. Res. Results, Northeastern Ser., No. 1 (1978).
8 Redfern, R.E., Warthen, J.D. Jr, Mills, G.D. Jr, and Uebel, E.C., USDA, Agric. Res. Results, Northeastern Ser., No. 5 (1979).
9 Rembold, H., and Schmutterer, H., in: Regulation of Insect Development and Behaviour, vol. 2, p. 1087. Eds F. Sehnal, A. Zabza, J.J. Menn and B. Cymborowski, Wroclaw Techn. Univ. Press, Wroclaw 1981.
10 Kubo, I., and Klocke, J.A., Agric. biol. Chem. *46* (1982) 1951.
11 Rembold, H., Sharma, G.K., Czoppelt, Ch., and Schmutterer, H., Z. ang. Ent. *93* (1982) 12.
12 Sieber, K.-P., and Rembold, H., J. Insect Physiol. *29* (1983) 523.
13 Rembold, H., and Sieber, K.-P., Z. Naturforsch., Ser.C *36* (1981) 466.
14 Redfern, R.E., Kelly, T.J., and Hayes, D.K., in: Juvenile Hormone Biochemistry, p. 415. Eds G.E. Pratt and G.T. Brooks. Elsevier/North Holland, Amsterdam 1981.
15 Lazaroviçi, P., and Pener, M.P., Gen. comp. Endocr. *33* (1977) 434.
16 Pener, M.P., Troetschler, R., Fridman-Cohen, S., Zeldes, I., Nassar, S.G., and Staal, G.B., in: Regulation of Insect Development and Behaviour, vol. 1, p. 357. Eds F. Sehnal, A. Zabza, J.J. Menn and B. Cymborowski. Wroclaw Techn. Univ. Press, Wroclaw 1981.
17 Wajc, E., and Pener, M.P., Israel J. Zool. *18* (1969) 179.
18 Pener, M.P., in: Proc. Int. Study Conf. Current Future Problems Acridology, p. 135. Eds C.F. Hemming and T.H.C. Taylor. Centre for Overseas Pest Research, London 1972.
19 Pener, M.P., Fridman-Cohen, S., and Levy, A., in: Juvenile Hormone Biochemistry, p. 323. Eds G.E. Pratt and G.T. Brooks. Elsevier/North Holland, Amsterdam 1981.
20 Cantacuzène, A.-M., and Girardie, A., C.R. hebd. Seanc. Acad. Sci. Paris, Ser.D *270* (1970) 2465.
21 Miall, R.C., J. Insect Physiol. *26* (1980) 607.
22 Kutsch, W., in: Physiology and Biophysics of Insect Flight, Special Aspects II. p. 71. Ed. W. Nachtigall. Biona Report 2. Fischer-Verlag, Stuttgart 1983.
23 Pener, M.P., Acrida *5* (1976) 189.

0014-4754/84/121418-03$1.50 + 0.20/0

Aldose metabolism in developing human fetal brain and liver[1]

B.K. Samanta, N.C. Chandra, S. Ghosh and K.L. Mukherjee[2]

Department of Biochemistry, Institute of Post Graduate Medical Education and Research, Calcutta 700020 (India), and Department of Biochemistry, Institute of Child Health, Calcutta 700017 (India), 20 September 1983

Summary. Aldose reductase, sorbitol dehydrogenase, and glucose-6-phosphate dehydrogenase enzyme activities were studied in human foetal brain and liver at different periods of gestation. Aldose reductase activity in liver disappears after 16 weeks of gestation whereas sorbitol dehydrogenase keeps on increasing in liver as well as in brain. In utero, some glucose metabolism may be mediated through an active sorbitol pathway in human fetuses.

Key words. Fetal development; brain, human; liver, human; aldose metabolism; sorbitol pathway.

In most mammalian species fructose is the predominant sugar in intrauterine life[3-7]. Biotransformation of glucose to fructose takes place in mammalian nerve and brain[8-11], lens[12,13], seminal vesicle[14], and sheep fetal liver[15]. Under the relatively anaerobic conditions prevailing in human intrauterine life[16,17], the permeation and metabolism of glucose in brain may follow alternate pathways. Hence it is of importance to study the conversion of glucose to fructose in human fetal tissues. We have measured the enzymatic activities of aldose reductase (alditol: $NADP^+$ 1-oxidoreductase, E.C. 1.1.1.21) and sorbitol dehydrogenase (SDH, L-iditol: NAD oxidoreductase, E.C. 1.1.1.14) in human fetal brain and liver tissues. The first enzyme catalyses the reduction of a variety of sugars to the corresponding polyols in a $NADP^+$ linked reaction:

$$\text{Aldose}+\text{NADPH}+\text{H}^+ \rightleftharpoons \text{Polyol}+\text{NADP}^+$$

The second enzyme catalyses the reaction:

$$\text{Sorbitol}+\text{NAD}^+ \rightleftharpoons \text{Fructose}+\text{NADH}+\text{H}^+$$