Nutritional Effects on Vitellogenesis in Species of *Drosophila*[1]

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J. Entomol. Sci. 35(4): 452-464 (October 2000)

Abstract A comparative study of vitellogenesis in response to feeding was made in females of three species of the genus *Drosophila,* belonging to different species groups. The process of vitellogenesis, although conservative, shows significant differences in species phylogenetically distant to *D. melanogaster* (Meigen). Females of *D. virilis* (Sturtevant) and *D. hydei* (Sturtevant) enter vitellogenesis and produce mature eggs only after proteinaceous food intake, but not under sucrose diet, while *D. melanogaster* females are able to support oocyte development upon maturity under sucrose diet. Electrophoretic analysis of the hemolymph of *D. virilis* and *D. hydei* starved or sucrose-fed females shows yolk protein synthesis at trace amounts. Interestingly, in the absence of proteinaceous food one or two other polypeptides of slightly higher molecular mobility than vitellogenins (but not vitellogenins) are produced, indicating a condition of starvation shock. These polypeptides are not detected in the hemolymph of either starved or sucrose-fed females *D. melanogaster* in which the vitellogenins are produced at low rate or significant amount, respectively. The results indicate that starvation affects ovarian yolk protein synthesis as well. Conversely, starvation does not directly affect the process of yolk protein uptake by the developing oocytes.

Key Words *Drosophila,* vitellogenesis, yolk proteins, starvation, sucrose diet

Accumulation of yolk (vitellogenesis) is a central event in the reproductive maturation of adult insects females. During this process the fat body produces yolk protein precursors, called vitellogenins, that are released and circulate in the hemolymph (Kunkel and Nordin 1985, Raikhel and Dhadialla 1992, Raikhel and Snigirevskaya 1998), from where they are selectively sequestered into the developing oocytes by endocytosis (Giorgi and Jacob 1977). In *Drosophila,* two or three vitellogenins are synthesized in the fat body depending on the species (Postlethwait and Jowett 1980, Bownes 1982, Hatzopoulos and Loukas 1986, Lamnissou 1996) and are transported to the ovary, under hormonal control, via hemolymph (Bownes 1986). The best studied dipteran vitellogenins are the three major yolk proteins of *D. melanogaster* (Meigen) designated YP1, YP2, YP3 (or VG1, VG2, VG3, when circulating in the hemolymph). These proteins are synthesized mainly in the fat body (Bownes and Hames 1977), but also in the ovarian follicle cells and transported directly into the oocytes (Warren and Mahowald 1979, Isaak and Bownes 1982, Brennan et al 1982) through inter-follicular spaces (Srdic et al. 1979, Postlethwait et al. 1980). The yolk proteins are related to each other and are encoded by single copy genes located on the X chromosome (Barnett et al. 1980). The regulation of the expression of yolk protein

[1]Received 07 September 1999; accepted for publication 06 February 2000.

genes in the fat body is complex involving ecdysone and juvenile hormone (Bownes 1986). Moreover, a nutritional regulation of yolk-protein gene expression in the fat body has been shown. Bownes et al. (1988) demonstrated that, in *D. melanogaster,* the yolk protein genes are continuously transcribed in the fat body of females from the time of eclosion and as long as food is available. When females are starved the yolk protein genes are transcribed at low levels, whereas, subsequent feeding stimulates increased transcript levels and, hence, increased yolk protein synthesis (Bownes et al. 1988). It also has been shown that in *D. melanogaster* the effects of starvation can be partially overcome by sucrose. More recently, Sondergaard et al. (1995) suggested that nutrition modifies the level of a trans-acting factor in the fat body. Induction of vitellogenesis by protein rich food also has been observed in other Diptera (i.e., *Aedes*) (Van Handel and Lee 1984, Koller and Raikhel 1991), *Musca* (Adams and Gerst 1992, 1993), *Phormia* (Zou et al. 1988), as well as in other insects such as *Rhodnius* (Valle et al. 1992, Guerenstein and Nunez 1994), *Triatoma* (Guerenstein and Nunez 1994), and *Spilostethous* (Ibanez et al. 1993).

In this paper, I report on the initiation of vitellogenin synthesis and ovarian development in response to feeding in different species of the fruit fly *Drosophila.* The study examines the effect of feeding a complete diet, sucrose, or only water (starvation) on these main processes of egg production during the adult life in species phylogenetically distant from each other to determine whether the mechanism of nutritional regulation of vitellogenesis is conservative. Three species of the genus *Drosophila* belonging to different species groups were used. The species *D. melanogaster* belongs to the *melanogaster* group and was used as a control. The two other species, *D. virilis* and *D. hydei,* are phylogenetically distant to *melanogaster* species group belonging to the *virilis* phylad and *mulleri* group, respectively.

Materials and Methods

Laboratory cultures of *D. hydei* and *D. virilis,* obtained from National *Drosophila* Resource Centre (OH, USA), were used in this study. Moreover, *D. melanogaster* from our laboratory culture was used for control experiments. The cultures were maintained on standard yeast, sugar, agar, cornmeal and tomato medium, at 23°C, under a photoperiod of 12:12h light, dark. Adult flies were synchronized at eclosion, within a period of 5 min.

Three categories of females were used in this study for each of the three species: (1) newly-eclosed females were transferred to vials containing fresh *Drosophila* food where they remained for 2 to 3 d, depending on the experiment and the species, (2) newly-eclosed females were transferred to vials containing agar-sucrose food where they remained for 2 to 10 d, depending on the experiment (flies were able to stay alive for more than 10 d), (3) newly-eclosed females were transferred to vials containing only agar, where they remained for 2 or 3 d depending on the species. Female *D. virilis* and *D. hydei* were able to live under starvation for 3 (or even more) days, but females of *D. melanogaster* remained alive only 2 to 2.5 d.

A number of starved, sucrose-fed, and complete food-fed females from the three species was dissected 2 or 3 d after eclosion, depending on the species. Their ovaries were examined under the microscope. Newly-eclosed *D. virilis* and *D. hydei* females were kept on sucrose diet for 5 or 10 d before dissection. The maximum developmental stage (MDS) of the oocytes observed in the ovaries of each female was recorded using the developmental stages established by King (1970).

Hemolymph from females of the three previously described categories of *D. melanogaster, D. virilis* and *D. hydei* was collected by opening the thoraces of a number of individuals, as described elsewhere (Lamnissou 1996). The wounded females were kept on the top of the vials using glasswool and centrifuged immediately at 3000 × g, for 5 min, at 4°C. The hemolymph was collected in small vials and stored at −20°C. Equal volumes of hemolymph (1 µl) were boiled for 7 min in 30 µl of buffer consisting of Tris-HCl, pH 6.8, 3% β-mercaptoethanol, 10% glycerol, 0.002% bromophenol blue and used for electrophoretic analysis. Samples of hemolymph from complete food-fed (control), sucrose-fed and starved females of the three species were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis on 8% slab gels (Laemmli 1970). The gels were stained in 0.1% solution of coomassie brilliant blue in 50% methanol, 7.5% acetic acid, and destained in 10% methanol, 7.5% acetic acid.

To identify whether polypeptides circulating in the hemolymph with slightly different mobility than the normal YPs are vitellogenins, ovarian and hemolymph samples from starved *D. virilis* and *D. hydei* females were resolved by SDS-PAGE analysis. Proteins were then electrophoretically transferred (Trans-Blot^{R} SD Semidry Transfer Gel, BIO-RAD) to nitrocellulose membranes in transfer buffer containing 40mM Glycine, 50mM Tris, 0.04 SDS, 20% methanol. Following transfer, blots were blocked directly for 1 h in 10% nonfat powdered milk, dissolved in PBS-T (0.1% Tween 20, 150mM NaCl, in 10 mM PBS pH 7.2) and subsequently incubated with anti-YP serum diluted 1:3000 in blocking buffer for 1h. The anti-YP serum used in this study was antiserum to the isolated *D. melanogaster* major egg YPs that was raised in New Zealand white male rabbits, as previously described by Trougakos et al. (1999). After washing in PBS-T, blots were incubated for 1 h with the secondary antibody [donkey anti-rabbit IgG/HRP conjugated (Amersham; NA-934)] diluted 1:8000 in blocking buffer; washed with PBS-T and PBS and developed with DAB solution (0.03%H202, 0.6% diaminobenzidin in 50mM Tris-HCl, pH 7.6).

To investigate the role of feeding on yolk protein uptake by the developing oocyte, females were maintained on fresh food for 2 d, and subsequently transferred to vials without food, where they remained for 3 d. Then, females were dissected and the MDS of the ovarian oocytes was recorded. Obviously, females remained on food for an appropriate length of time to synthesize yolk proteins, but not a sufficient length of time to mature eggs. In order to establish this time, a number of fed *D. virilis* and *D. hydei* females of different age were dissected and the development of their ovaries was examined.

Results and Discussion

Ovarian development of *Drosophila,* either starved or fed on sucrose or complete *Drosophila* medium, was variable from one experiment to another. Bownes and Blair (1986), who studied the effects of dietary components on yolk protein transcription in *D. melanogaster,* observed the same. In *D. melanogaster,* the majority of the 2-d-old females examined starved from eclosion, had rudimentary previtellogenic ovaries (Table 1, Fig. 1A), while 11.3% of these females had vitellogenic ovaries containing a small number of mature oocytes (Fig. 1B). Conversely, *D. melanogaster* females of the same age, maintained from eclosion on sucrose medium or on complete *Drosophila* medium had vitellogenic ovaries (with the exception of a few sterile females) containing stage-14 mature oocytes (Fig. 1C, D; Table 1). The SDS-PAGE analysis

Table 1. Ovarian development in starved, sucrose-fed and complete food-fed, 2-d-old *D. melanogaster* females. n: number of females examined, pre: females with previtellogenic ovaries, vitel: females with vitellogenic ovaries

	n	Ovarian development		% Females with mature eggs
		pre	vitel	
starved	62	55	7	11.29
sucrose	43	4	39	90.69
complete food	20	1	19	95.00

of equal volumes of hemolymph of *D. melanogaster* females from the two dietary categories (sucrose-fed and starved females) is shown in Fig. 2. I used the ovarian sample together with the hemolymph samples in order to verify the position of the major yolk proteins. The ovarian extract of *D. melanogaster* reveals the existence of three quantitatively enriched major polypeptides (YP1, YP2, YP3). The sample of hemolymph of 2-d-old sucrose-fed *D. melanogaster* females has significant amounts of the three major yolk proteins or vitellogenins VG1, VG2, VG3. However, starvation reduces hemolymph vitellogenin levels in adult females. Equal volumes of hemolymph of starved females show only trace amounts of vitellogenins (Fig. 2).

Ovarian development and electrophoretic analysis of hemolymph from starved and sucrose-fed *D. virilis* and *D. hydei* females exhibited significant differences compared with those observed in *D. melanogaster.* In *D. virilis* starvation affects egg production more dramatically. Among 72 starved *D. virilis* females examined 3 d after eclosion, about 99% had rudimentary previtellogenic ovaries (Fig. 1E). In a few cases, only trace amounts of yolk were observed in some oocytes (Fig. 1F). Similar results were observed in sucrose-fed *D. virilis* females. With the exception of a low number of females (5%) that produced mature eggs, the majority of sucrose-fed *D. virilis* females had previtellogenic ovaries (Table 2). Conversely, *D. virilis* females maintained on complete medium from eclosion had mature ovaries containing stage 14 oocytes (Fig. 1G). The mean number of mature oocytes, as well as vitellogenic stages 9-13 oocytes, for each category of female, is recorded in Table 2. I must mention that the numbers presented are based only on females that entered the vitellogenic stages 9-14. It is clear that starvation or sucrose diet dramatically affects vitellogenesis and, hence, egg production in this species. Contrary to *D. melanogaster, D. virilis* females fed the sucrose diet generally cannot support vitellogenesis to egg maturity. In order to test whether starvation or sucrose diet from eclosion affects fat body vitellogenin synthesis, I analyzed by SDS-PAGE the hemolymph of 3-d-old *D. virilis* females. Fig. 3 shows the electrophoretic analysis of ovarian extract and hemolymph of complete medium-fed *D. virilis* females as well as equal volumes of hemolymph of sucrose-fed and starved *D. virilis* females. Two vitellogenins or yolk proteins, YP1 and YP2 (the first one being heavily stained probably, consisting of two polypeptides), are present in both ovarian extract and hemolymph of 3-d-old complete food-fed females. These two yolk proteins cannot be detected in hemolymph of either sucrose-fed or starved females. However, two other polypeptides with slightly higher molecular weights are

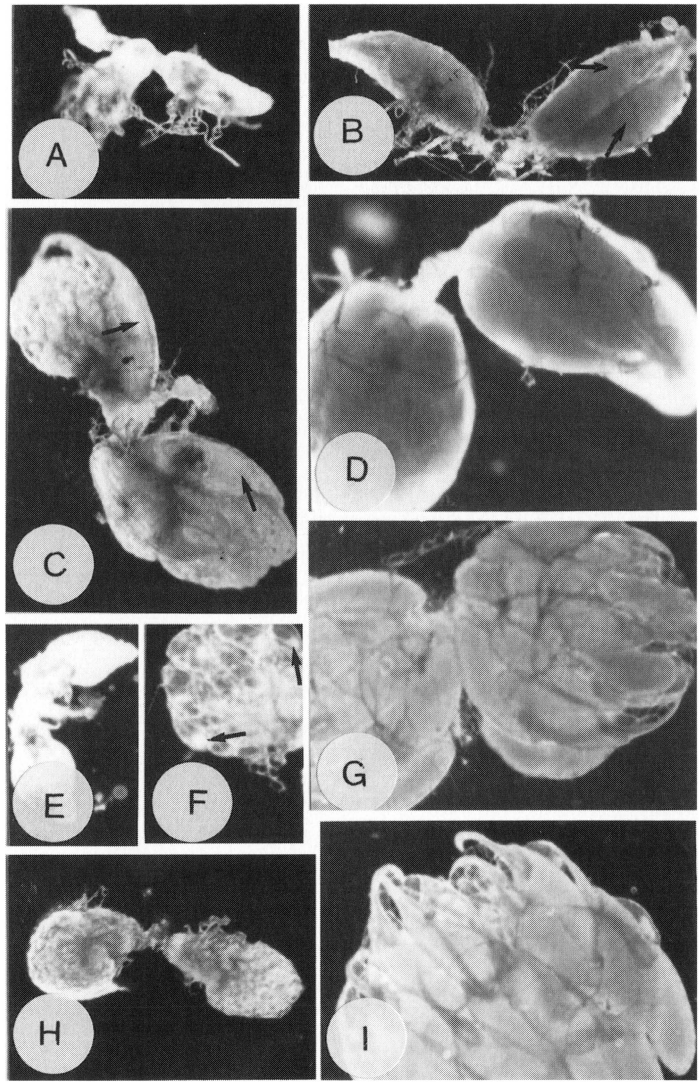

Fig. 1. Ovarian development of complete food fed, sucrose-fed and starved females of *D. melanogaster, D. virilis, D. hydei* (×100). (A) previtellogenic ovaries of starved *D. melanogaster* female, (B) vitellogenic ovaries of starved *D. melanogaster* female showing mature oocytes (arrows), (C) vitellogenic ovaries of sucrose-fed *D. melanogaster* female showing mature oocytes (arrows), (D) vitellogenic ovaries of complete food-fed *D. melanogaster* female showing a number of mature oocytes, (E) previtellogenic ovaries of starved *D. virilis* female, (F) ovary of starved *D. virilis* female showing trace amounts of yolk (arrows), (G) mature ovaries of complete-food fed *D. virilis* female, (H) previtellogenic ovaries of sucrose-fed *D. hydei* female, (I) mature ovary of complete food-fed *D. hydei* female.

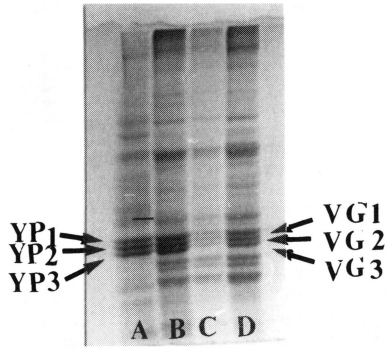

Fig. 2. SDS-PAGE analysis on 8% slab gels of ovarian extract (A) and equal amounts (1 µl) hemolymph of complete food-fed (B), starved (C) and sucrose-fed (D) 2-d-old *D. melanogaster* females. The major yolk proteins YP1, YP2, YP3 of ovarian extract as well as the major vitellogenins, VG1, VG2, VG3, of the hemolymph are indicated with arrows.

Table 2. Ovarian development in starved, sucrose-fed and complete food-fed, 3-d-old *D. virilis* females. n: number of females examined, pre: females having previtellogenic ovaries

	n	pre	% Females with s14 oocytes	Number of s9-13 oocytes per female*	Number of s14 oocytes per female*
starved	72	66	1.38	5	3
sucrose fed	59	56	5.08	4	5
complete food	30	1	93.33	11	14

* The numbers presented are the means based on females enter vitellogenesis and reach the vitellogenic stages 9-14.

observed in hemolymph of both sucrose-fed and starved *D. virilis* females (Fig. 3). These polypeptides can be correlated with the absence (or low-rate synthesis) of the major vitellogenins and must be either polypeptides that are produced, instead of vitellogenins, under starvation shock or, they might be some forms of vitellogenins having higher molecular weight than VG1 and VG2. In either event these polypeptides exist only when no vitellogenins are observed (Fig. 3).

Morphological observations of ovarian development in starved *D. hydei* females show the lack of development beyond previtellogenic stages (Table 3). The ovaries of starved females seldom contained trace amounts of yolk. None of 3-d-old *D. hydei* females, starved from eclosion, contained mature oocytes (Table 3). Similar results have been observed in sucrose-fed *D. hydei* females. About 94% of the examined females were unable to develop ovaries upon maturity (Fig. 1H). Conversely, complete medium-fed females of the same age produced mature oocytes (Fig. 1I). Gel

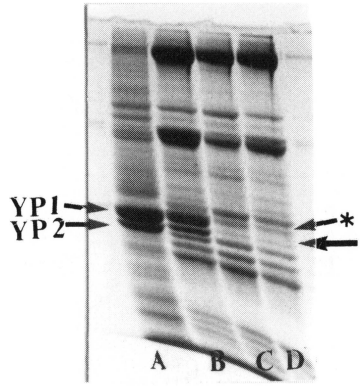

Fig. 3. SDS-PAGE analysis on 8% slab gels of ovarian extract (A) and equal amounts (1 μl) of hemolymph of complete food-fed (B), sucrose-fed (C), and starved (D) 3-d-old *D. virilis* females. The major yolk proteins, YP1, YP2, are indicated. Arrow shows the absence of VG1 and VG2. *Arrow shows the presence of other polypeptides in the samples C and D.

Table 3. Ovarian development in starved, sucrose-fed and complete food-fed, 3-d-old *D. hydei*. n: number of females examined, pre: females having previtellogenic ovaries

	n	pre	% females with s14 oocytes	Number of s9-13 oocytes per female*	Number of s14oocytes per female*
starved	76	69	0	2	0
sucrose fed	65	58	7.70	4	3
complete food	30	0	96.66	10	15

* The numbers presented are the means based only on females enter the vitellogenic stages 9-14.

electrophoresis of total proteins present in the hemolymph of females of the three dietary groups is shown in Fig. 4. The ovarian extract and hemolymph of 3-d-old complete food-fed *D. hydei* females contain 3 yolk proteins, YP1, YP2, YP3, which do not exist in the hemolymph of starved or sucrose-fed females (Fig. 4). Similar to previously described results with *D. virilis*, in the absence of YPs, another polypeptide of slightly different motility, with higher molecular weight, is detected in the hemolymph of sucrose-fed and starved *D. hydei* females (Fig. 4).

In order to verify whether the polypeptides with slightly higher molecular weight than the major vitellogenins, circulating in the hemolymph of starved *D. virilis* or *D. hydei* females are yolk proteins, immunoblotting analysis using *D. melanogaster* YPs antibody was conducted. A prominent positive cross-reaction of the antibody occurred at the position of YP1 and YP2 in the ovarian extract samples of complete food-fed *D.*

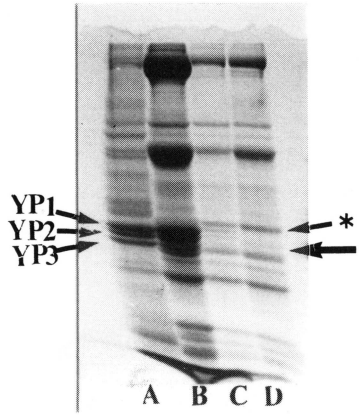

Fig. 4. SDS-PAGE analysis on 8% slab gels of ovarian extract (A), and equal amounts (1 µl) of hemolymph of complete food-fed (B), sucrose-fed (C), and starved (D) 3-d-old *D. hydei* females. The major yolk proteins, YP1, YP2, YP3, are indicated. Arrow shows the absence of VG1, VG2 and VG3. *Arrow shows the presence of other polypeptide in the samples C and D.

virilis and *D. hydei* females (Fig. 5A,D), but no cross reaction occurred in hemolymph samples (Fig. 5B-E), at the position where the unknown polypeptides of higher molecular weight migrate. Obviously, these unknown polypeptides are not vitellogenins, but they are some other polypeptides produced during starvation stress. Moreover, the ovarian extract of starved *D. virilis* (Fig. 5C) and *D. hydei* (Fig. 5F) females do not show any reaction with the *D. melanogaster* YPs antibody, confirming the previously described morphological observations showing that ovaries from starved (or sucrose-fed females) do not enter vitellogenesis. However, a faint cross reaction is seen in the

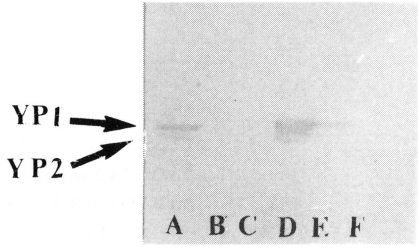

Fig. 5. Immunoblot analysis, using as a probe the antiserum against the *D. melanogaster* YPs, of (A) ovarian extract of complete food-fed mature *D. virilis* female, (B) hemolymph of 3-d-old starved *D. virilis* females, (C) ovarian extract of 10 starved *D. virilis* females, (D) ovarian extract of complete food-fed mature *D. hydei* female, (E) hemolymph of 3-d-old starved *D. hydei* females, (F) ovarian extract of 10 starved *D. hydei* females.

sample of hemolymph of starved *D. hydei* females (Fig. 5E), indicating a low rate vitellogenin synthesis under starvation.

The flies are able to live for days on the sucrose diet. In order to examine whether these females enter vitellogenesis and develop mature oocytes, probably later than the control females, newly-eclosed females of the two species were fed sucrose diet, for 5 and 10 d and were then examined for ovary maturation. No ovarian development was observed suggesting that, in contrast to *D. melanogaster, D. virilis* and *D. hydei,* need complete proteinaceous food to mature eggs (Table 4).

The previous experiments show that in *D. virilis* and in *D. hydei* starved or sucrose-fed females, fat body is unable to synthesize vitellogenin, but the effect of starvation on the process of vitellogenin uptake by the developing oocyte is not understood. In order to examine whether starvation affects uptake, as well, I studied ovarian development of females which were maintained on complete medium from eclosion for 2 d and then transferred to starvation conditions for another 3 d. A number of females of each species was dissected before and after starvation to determine ovarian development. Females examined before transfer off food had either previtellogenic ovaries or ovaries containing oocytes in early vitellogenic stages (Table 5). Morphological observations after 3 d of starvation demonstrated progressive ovary development upon maturity. Maximum developmental stage after starvation, in both species not only was increased but reached the final developmental stage 14 (Table 5). Clearly, the yolk proteins circulating in the hemolymph were able to enter the developing oocytes during the period of starvation and, thus, the oocytes completed development up to stage 14. These results suggest that starvation does not, directly, affect the process of vitellogenin uptake by developing oocytes.

Comparative studies of ovarian development from starved females of *D. melanogaster* (Table 1), *D. virilis* (Table 2), and *D. hydei* (Table 3) show differences between the first and the last two species suggesting that starvation affects egg production in a direct way, in *D. virilis* and *D. hydei.* Conversely, a number of starved *D. melanogaster* females were able to develop oocytes upon maturity (Fig. 1B). Autogeny (vitellogenesis and egg production without feeding) occurs in about 11% of starved *D. melanogaster* females, but in very low rates in *D. virilis* (1.3%) (Table 2) and *D. hydei* (0%) (Table 3) starved females. However, autogeny has been reported in a number of species (Chinzei and Taylor 1994, Feldman-Muhsam 1973) and is believed to be

Table 4. Ovarian development in sucrose-fed *D. virilis* and *D. hydei* females n: number of females examined, pre: females having previtellogenic ovaries

Species	n	Age*	pre	% females with s14 oocytes	Number of s14 oocytes per female**
D. virilis	22	5	22	0	0
D. virilis	40	10	39	2.56	2
D. hydei	28	5	26	7.14	2
D. hydei	38	10	36	5.26	3

* In days.

** The numbers presented are the means based on females having mature eggs.

Table 5. Ovarian development of *D. virilis* and *D. hydei* females which remained from eclosion on food for 2 d (*) and then were transferred in starving conditions for 3 d (**). MDS: Maximum Developmental Stage of oocyte development, N: number of oocytes

Species	MDS	N of s9 per female†	N of s10 per female†	N of s11 per female†	N of s14 oocytes per female†
*D. virilis**	pre or 8-10	11	7	0	0
*D. virilis***	14	6	5	1	35
*D. hydei**	8-11	12	4	1	0
*D. hydei***	14	7	4	0	28

† The numbers presented are the means based on 30 females.

critical for progeny production in species that are not likely to feed as adults. Furthermore, the results reported in this study indicate that dietary food, such as sucrose medium, is well accepted by *D. melanogaster* (Table 1) but, surprisingly, not by *D. virilis* (Table 2) and *D. hydei* (Table 3). It seems, therefore, that in difference to *D. melanogaster,* sucrose diet is poor food to support vitellogenesis in *D. virilis* and *D. hydei.*

Drosophila vitellogenins are released in the hemolymph immediately after their synthesis in the fat body (Johnson and Butterworth 1985, Bownes 1986). However, the results of this study show no main vitellogenins in *D. virilis* and *D. hydei* females starved or fed on sucrose diet (Fig. 3,4). Instead, in the absence of proteinaceous food, *D. virilis* and *D. hydei* females produce one or two polypeptides of slightly higher molecular weight than vitellogenins. These are not vitellogenins because they show no reaction with the *D. melanogaster* anti-YPs antibody (Fig. 5). Clearly, the production of these polypeptides is due to a starvation stress. Furthermore, it appears that when diet is poor, flies cease egg production to keep reserves for other functions needed to maintain adult survival. Thus, it is clear from these results that, contrary to *D. melanogaster* where vitellogenin synthesis can be occur in the absence of proteinaceous food, proteinaceous food is absolutely necessary for vitellogenesis in *D. virilis* and *D. hydei.*

Several studies in *D. melanogaster* have indicated that the ovaries synthesize significant amounts of yolk proteins (Srdic et al. 1979, Postlethwait et al. 1980, Brennan et al. 1982). These yolk proteins are synthesized in the ovarian follicle cells and do not enter and circulate in the hemolymph, but are incorporated directly by the oocytes (Srdic et al. 1979, Postlethwait et al. 1980). Obviously, the yolk proteins of ovarian origin do not contribute to levels found in the hemolymph. The results of this study showed that the ovaries of starved or sucrose-fed *D. virilis* and *D. hydei* females do not have vitellogenic oocytes, even 10 d after eclosion, for sucrose-fed females (Table 4), suggesting that either starvation or sucrose diet affects ovarian yolk protein synthesis, as well, or that the synthesis of yolk proteins of follicular origin starts synchronously or after the absorption of the vitellogenins from the hemolymph by the developing oocyte.

The results of this study further demonstrate that, in *Drosophila,* the initiation of uptake of yolk proteins by the oocytes is not dependent on feeding. Accumulation of vitellogenins in the *Drosophila* oocyte occurs during the developmental stages 8-10. During the latter part of oogenesis (stages 10-14) the follicle cells secrete the complex egg envelopes (choriogenesis) around each oocyte (King 1970, Margaritis et al. 1980). Results presented in this study show that females possessing previtellogenic oocytes or oocytes of stages 8-9 were able to complete both vitellogenesis and choriogenesis, during a period of starvation. Thus, it is clear from these results that, in *D. virilis* and *D. hydei,* starvation does not directly affect the process of uptake by the developing oocytes. Furthermore, starvation does not affect the process of choriogenesis. Bownes and Blair (1986) reported that when older *D. melanogaster* females are starved, the ovary ceases uptake of yolk and further egg development, although circulating yolk-protein levels, remain high.

Nutrition is obviously a major step in the process of vitellogenin synthesis in the fat body and ovarian follicle cells of *Drosophila,* but it does not directly affect the process of uptake. It is known that the process of vitellogenesis in insects is conservative. Even though *D. virilis* and *D. hydei* are phylogenetically distant to *D. melanogaster,* it is surprising that they do not have an equal response to sucrose medium. Our results clearly show that *D. virilis* and *D. hydei* are absolutely dependent on proteinaceous food to initiate vitellogenesis and produce mature eggs. The suggestion by Bownes and Reid (1990) that the presence of a signal from the *D. melanogaster* gut that acts on the fat body and the ovary to initiate this process appear logical.

The role of feeding and nutrition on egg development in some hematophagous and other insects has been documented. In the anautogenous mosquito, *Aedes aegypti* (L.), it is known that both yolk protein synthesis and vitellogenin endocytosis are first activated as a result of a blood meal (Raccioppi et al. 1984, Van Handel and Lea 1984, Koller and Raikhel 1991). In *Rhodnius,* juvenile hormone is normally released following a blood meal and initiates vitellogenesis (Davey 1981, Guerestein and Nunez 1994). In the housefly, *Musca domestica* (L.), protein feeding is required for ovarian maturation (Adams and Nelson 1990, Adams and Gerst 1991). When a complete diet was fed to houseflies that where maintained on sucrose, both hemolymph ecdysteroid and vitellogenin concentrations increased and the first cycle of ovarian follicles matured (Adams and Gerst 1991). Similar results were observed for the second cycle of oogenesis in this insect. It seems that in *M. domestica* feeding on a nutritionally adequate diet provided materials that were required for steroidogenesis and vitellogenesis (Adams and Gerst 1993). The gut hypothesis suggested by Bownes and Reid (1990) for *Drosophila* seems to fit well the data from *Musca.* In *Phormia regina* (Meigen) feeding on protein induced an endocrine cascade leading to elevated ecdysteroid levels (Yin et al. 1990) and vitellogenin concentration that initiates ovarian development (Zou et al. 1988). Furthermore, the effect of starvation on hemolymph vitellogenins and ovary uptake was demonstrated in *Spilostethus pandurus* (Scop.) by Ibanez et al. (1993) indicating also, the strong role of the juvenile hormone as the regulatory factor of both reproductive phenomena in this species. Contrary, in *Drosophila* it has been reported that the nutritional status does not appear to affect ecdysone or juvenile hormone (Bownes and Reid 1990), but the fact that these hormones can increase yolk protein synthesis in starved flies suggests that vitellogenesis is either inhibited or not activated in starved flies.

I may conclude that, in the fruit fly *Drosophila,* nutrition is a major step in the process of vitellogenin synthesis in the fat body and ovarian follicle cells, but it does

not directly affect the process of uptake. It is clear that during evolution of the genus *Drosophila* the production of eggs in the phylogenetically-newer species, *D. virilis* and *D. hydei,* became dependent on protein feeding. Thus, in *Drosophila,* as well as in some other insects, protein feeding acts as a trigger that induces vitellogenin synthesis and ovarian development.

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