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Vitellin is the nutrient reserve during starvation in the nymphal stage of a tick

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Summary. In the tick *Ornithodorus moubata,* the major protein of egg yolk, vitellin, was conserved in the guts of larvae and nymphs in aggregated forms for over four months after hatching. Vitellin was the nutrient which supported tick survival until the nymph could obtain a blood meal. This adds to the known role of yolk protein as the nutrient reserved for embryos a new role as a reserve for post-embryonic development and during starvation in the nymphal stage.
Key words. Tick; *Ornithodoros moubata;* embryogenesis; vitellin; egg yolk; nutrient reserve; nymphal stage; starvation.

The major proteins of egg yolk – vitellin (Vn) in insects and other invertebrates, lipovitellin and phosvitin in oviparous vertebrates – are generally considered to be nutrient reserves for embryonic development. The synthesis of their precursors, vitellogenins (Vg) in the fat body or liver, respectively, provides good material for the study of the control of specific gene activation by hormones[1-4]. The physiological roles and eventual fate of these proteins, on the other hand, has been relatively little investigated. In certain insects, Vn, which is a lipoglycoprotein, has been shown to be mostly consumed before hatching[5,6]. In Drosophila, three yolk polypeptides are digested during embryogenesis and are not detectable in the first instar larva[7]. In the silkworm, *Bombyx mori,* most of the Vn is used in the final stage of embryonic development and only a little remains in the first instar larva[8]. On the other hand, recently Vn was reported not to be an essential protein for silkworm embryogenesis[9]. To examine the role of Vn in another class of arthropod, I undertook a study of the fate and the essentiality of Vn during embryogenesis and further development in the soft tick, *Ornithodoros moubata* (sensu Walton 1962). I found that Vn is conserved over four months after hatching for use as the nutrient during starvation until the nymph can obtain a blood meal.

In this tick, the adult female synthesizes Vg in the fat body (Chinzei, unpublished data) and produces about 200 eggs after the stimulus of a blood meal. A female tick utilizes 75% of the blood meal protein for synthesis of Vg[10]. Vg purified from hemolymph of reproductive females was separated into two components (Vg1 and Vg2), identified as monomer (mol. wt 300,000) and dimer (600,000), respectively, by polyacrylamide

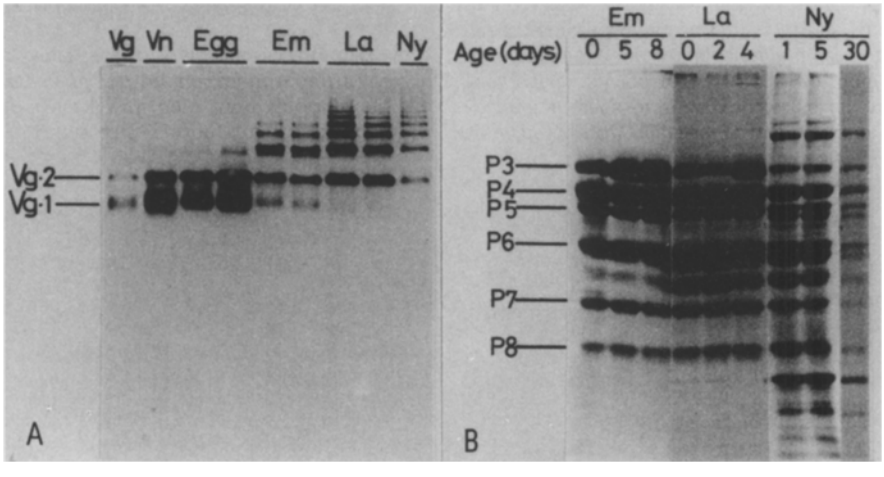

Figure 1. Polyacrylamide gel electrophoresis (PAGE) of soluble proteins from eggs, embryos (Em), larvae (La) and nymphs (Ny). Ticks were reared as described previously[11]. Eggs were collected within 24 h after oviposition and kept at 30 °C in batches laid on the same day. Ten eggs, larvae or nymphs were removed at definite intervals and were homogenized with a glass-teflon homogenizer in 5 vol. of extraction buffer (0.02 M Tris-HCl (pH 8.5), 0.4 M NaCl, 0.2% Triton X100, 0.2% SDS, 0.2% sodium deoxycholate, and 1 mM phenylmethyl sulfonyl fluoride). The homogenate was centrifuged at 5000 × g for 15 min at 4 °C. The supernatant crude extracts were analyzed by A) PAGE in 2.5 to 15% acrylamide gradient without SDS, and B) PAGE in 7% acrylamide with 0.1% SDS. Gels were stained with Coomassie blue R 250. Vg, Vn: vitellogenin and vitellin purified by anti-Vn-IgG affinity chromatography. See text for explanation of Vg1, Vg2 and P3 to P8.

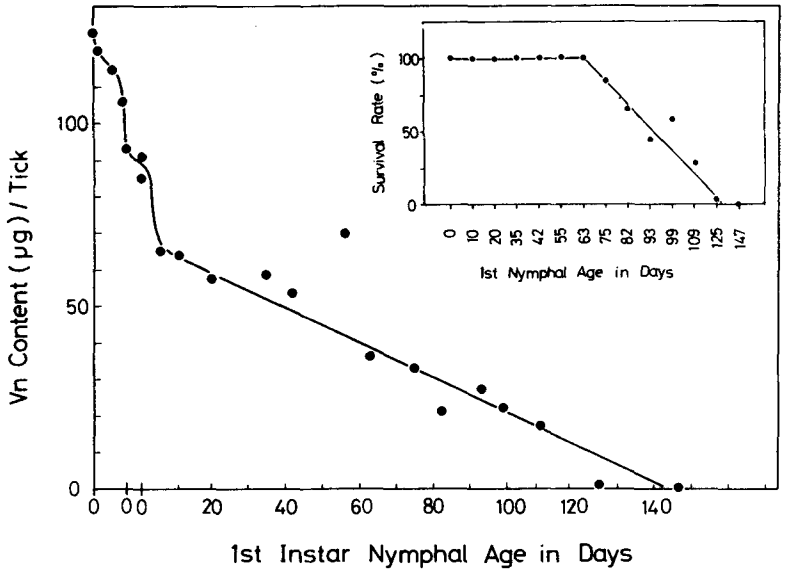

Figure 2. Changes of Vn content and survival rate during tick development. Ten eggs were taken from a batch laid on the same day and kept together at 30 °C. Animals developed almost synchronously. Survival in the nymphal stage was checked approximately every ten days for about five months (insert). The same samples of ticks were homogenized as described in figure 1. An aliquot of the supernatant was used for rocket immunoelectrophoresis and the Vn content in an egg, embryo, larva and nymph was determined by comparison with known amounts of purified Vn. Antiserum was prepared by injecting purified Vn into rabbits. Three zeros in the bottom scale show the day of oviposition, hatching and larval-nymphal ecdysis, respectively.

gel electrophoresis (PAGE), electron microscopy, gel filtration and immunodiffusion. A newly laid egg contains about 125µg Vn which is 80% of the total egg protein[10]. Crude egg extract and Vn isolated from eggs by anti-Vn-IgG Sepharose affinity chromatography revealed two bands equivalent to Vg1 and Vg2 (fig. 1A). Extracts from embryo, larva and nymph displayed a ladder-like electrophoresis pattern, most typically in the nymphal stage. All of the proteins composing the ladder were immunologically identical with Vn, as shown by an immunoreplication technique[11] (data not shown). Analysis of the crude larval extract by gel filtration chromatography on Bio-Gel A 1.5 m with reference proteins (not shown) demonstrated molecular weights of the peaks which are 8-, 6-, 4-, 2- and 1-fold that of the monomer Vg1, respectively. These data suggest that Vn aggregates to high mol.wt oligomers during embryogenesis and larval and nymphal development.

In analysis by PAGE with sodium dodecyl sulfate (SDS) six polypeptides (P3 to P8) were observed in the crude protein extracts from egg, embryo, larva and nymph (fig. 1B). P1 and P2 were specific polypeptides of the precursor Vg, and were not observed in these extracts[11]. Even 30 days after larval-nymphal ecdysis, the six polypeptides were detected in each extract approximately in the same ratio judging from the intensity of stained bands. The gels also showed several protein bands other than Vn components that are specific to embryo, larva or nymph.

Embryogenesis lasts 9–10 days at 30 °C. Larvae can ecdyse to nymphs in four days after hatching without a blood meal in this species, *O. moubata,* which is rather an exception among ticks. Nymphs, however, have to engorge blood from a vertebrate host once in order to ecdyse to the next stage. Nymphs in the 1st instar survive without a blood meal for two months at least and four months at most (fig. 2, insert) during this period, if they get a blood meal they can develop and ecdyse to second instar nymphs in about 10 days. To follow the utilization of the Vn stored in an egg, the quantities in eggs, embryos, larvae and nymphs were determined by rocket immunoelectrophoresis (fig. 2). From its initial level of about 125 µg, the Vn content dropped steeply in the final stage of embryogenesis before hatching and again at the larval-nymphal ecdysis. At the beginning of the nymphal stage about half of the original amount of Vn remained. Then, in the first instar nymphal stage the Vn content decreased at a slow, rather constant rate (approximately 0.46 µg/day) and reached zero at about 125 days after ecdysis. By this time, almost all nymphs were dead. Vn was detected in living ticks even after long starvation, but not immediately after death (data not shown). These data suggest that ticks can survive so long as Vn reserves remain, but no longer.

To localize Vn in the nymphal body immunohistochemically, sections of whole bodies at 10 days after ecdysis were stained indirectly with fluorescein isothiocyanate (FITC) labeled IgG. Strongly fluorescent material (Vn) was located in the lumen of the gut which occupies a large part of the body cavity and branches into many lobes (fig. 3). Integument, tracheae and some granules in the malpighian tubules or rectal gland gave weak fluorescence, because cuticle and excreta (guanine crystals) have an autogenous fluorescence under UV light (not

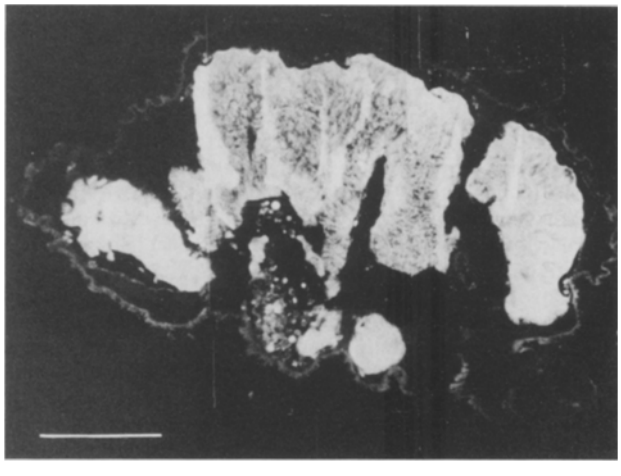

Figure 3. Immunofluorescent micrograph of nymphal whole body cross section. Nymphs were fixed in 90% ethanol and dehydrated with a series of ethanols (95–100%). Paraffin sections were prepared according to standard methods. All processes were carried out below 70 °C. 6-µm sections were cut. Sections were deparaffined with xylene, rinsed with a series of alcohols and then washed repeatedly with PBS. Tissue sections on a glass slide were overlaid with anti-Vn-IgG solution for 5 min at room temperature, and washed with PBS. Fluorescein isothiocyanate (FITC) conjugated anti-rabbit-IgG serum (goat) was diluted to one-tenth with PBS and applied to the tissue sections for 10 min. Preparations were rinsed again with PBS, and mounted in a drop of 1 M Tris-HCl:glycerol (1:9, v/v). They were observed and photographed with a Nikon microscope with an incident UV attachment and fluorescence optics. Scale: 200 µm.

shown). Strong fluorescence in the gut lumen was detected also in larvae and in nymphs over two months old.

The soft tick *O. moubata* in the first nymphal instar has to survive long periods of starvation until it encounters a vertebrate host. My findings indicate that Vn can support tick survival without food intake for some months. This adds to the known role of yolk protein as the nutrient reserve for embryos a new role as a reserve for post-embryonic development and during starvation in the nymphal stage.

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The mating behavior of individuals of *Drosophila pseudoobscura* from New Zealand

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Summary. Recently discovered individuals in New Zealand of the North American species *Drosophila pseudoobscura* were analyzed for any divergence in mating behavior. Cultures collected from five localities in North American were mated with a stock from Rotorua, New Zealand. No significant divergence was obtained in any of the within North Americ, and more importantly, between North American and New Zealand mating experiments. Further analyses also showed no development of sterility between recently caught New Zealand and North American flies. We discuss our results in relation to others of this type.
Key words. Drosophila pseudoobscura; mating behavior; divergence; sterility.

D. pseudoobscura is distributed over large areas of the west of North America. Until recently the only other population of *D. pseudoobscura* known outside this area, was a population near Bogotá, Colombia[3]. However Parsons[4] recently reported collecting a single female of *D. pseudoobscura* from Te Kaha in the East Cape of New Zealand. Lambert and McLea[5] despite extensive trapping in this area were unable to collect any individuals of this species at the same locality. However in a systemic study of the central North Island of New Zealand these authors located eight sites, of which one near Rotorua consistently yielded *D. pseudoobscura*.

The Bogotá population of *D. pseudoobscura* has been studied extensively. Prakash[6] found no excess of homogamatic matings when conducting multiple choice experiments among the Bogotá and three mainland North American populations. First generation males obtained from crossing Bogotá females to males of mainland populations were found to be sterile. The genetic basis of the sterility has been investigated and found to be complex, involving gene interactions and maternal effects. Early electrophoretic studies[7,8] showed reduced genetic variability and a lack of unique alleles in the Bogotá population, usually with the highest frequency mainland allele being fixed. However Singh et al.[9] using four different electrophoretic conditions and heat stability tests have reported an immense increase in the genetic variability from that recorded previously. These authors also revealed undetected electrophoretic differences between the mainland and the isolated Bogotá population.

A large number of studies have examined the mating behavior of individuals of geographically distinct populations of *Drosophila*. Of these, a significant number have reported changes in the mate recognition system of individuals. However this apparent trend is not found in all species of *Drosophila*, as shown by Henderson and Lambert[10]. Using 29 worldwide populations of *D. melanogaster* in 38 multiple choice experiments these authors detected only two cases of a significant deviation from random mating despite both morphological and genetic differentiation among the populations tested. These findings are consistent with the less extensive studies of Petit et al.[11] and Cohet and David[12]. Similar results have been obtained for populations of *D. pseudoobscura*[7,13,14]. Indeed both *D. melanogaster* and *D. pseudoobscura* are interesting species because of the remarkable stability exhibited by their mate recognition system.

Materials and methods. Details of collection dates, locality and number of females trapped are provided in table 1 and the figure for all stocks of *D. pseudoobscura* used. The populations were maintained at an effective population size of about 500 until mating and sterility experiments were conducted.

Table 1. Collection details of North American and New Zealand populations of *D. pseudoobscura*

Number	Locality	Date	Number of female flies collected
1	Placer Country, California	20/12/82	18–23
2	Palomar Mountain, California	6/ 8/83	25–30
3	Cave Junction, Oregon	25/ 5/83	12
4	Vancouver Island, British Columbia	9/ 8/83	12
5	Port Coguiltlam, British Columbia	3/ 9/83	12
6	Rotorua, New Zealand	11/ 2/82	52