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REVIEW

Unusual Functions of Insect Vitellogenins: Minireview

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Summary

Insect vitellogenins are an intriguing class of complex proteins. They primarily serve as a source of energy for the developing embryo in insect eggs. Vitellogenesis is a complex hormonally and neurally controlled process that command synthesis of vitellogenin molecules and ensures their transport from the female fat bodies or ovarial cells into eggs. The representatives of all insect hormones such as juvenile hormones, ecdysteroids, and neurohormones participate in vitellogenesis, but juvenile hormones (most insect species) and ecdysteroids (mostly Diptera) play the most important roles in the process. Strikingly, not only insect females, but also males have been reported to synthesize vitellogenins indicating their further utility in the insect body. Indeed, it has recently been found that vitellogenins perform a variety of biological functions in the insect body. They participate in defense reactions against entomopathogens such as nematodes, fungi, and bacteria, as well as against venoms such as the honeybee *Apis mellifera* venom. Interestingly, vitellogenins are also present in the venom of the honeybee itself, albeit their exact role is unknown; they most likely increase the efficacy of the venom in the victim's body. Within the bee's body vitellogenins contribute to the lifespan regulation as anti-aging factor acting under tight social interactions and hormonal control. The current minireview covers all of these functions of vitellogenins and portrays them as biologically active substances that play a variety of significant roles in both insect females and males, and not only acting as passive energy sources for developing embryo.

Key words

Vitellogenin • Defense reaction • Entomopathogen • Infection • Longevity

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Introduction

In most oviparous animals, vitellogenins serve as the building blocks for the proteins that make up the egg yolk. Insects are not an exception. The majority of vitellogenin's chemical and structural features, at least in the model insect organisms, have been fully elucidated. Generally, vitellogenins are energy-rich complexes of glycolipophosphoproteins with extra components coupled to the protein skeleton [1]. Most insect species synthesize two vitellogenins with a molecular mass between 210 and 650 kDa [1,2], however, there are rare exceptions to this rule such as some representatives of dipteran species.

Most insect species produce vitellogenins in their fat bodies, which are organs resembling the liver and adipose tissue in vertebrates. During vitellogenesis, the responsible fat body cells, called trophocytes, undergo significant cytological changes in an effort to transform from storage cells to specialized cells with high protein production. There are two primary phases they go through: previtellogenic and vitellogenic [4,5]. In the first phase, the activation and enlargement of nucleoli, the

growth of Golgi complexes, and the proliferation of ribosomes and rough endoplasmic reticulum are underway. In the following vitellogenic phase, a protein synthesis takes precedence over all organelle functions and energy sources. After the protein base is created, the vitellogenin molecules are phosphorylated or glycosylated, and then fatty components are joined. Consequently, vitellogenin molecules are synthesized in large quantities. Thereafter, these complexes are expelled into the haemolymph and carried to the ovaries – the main place of action, or, alternatively, to another location of need [6].

Role of vitellogenins in reproduction

Vitellogenins are a major nutritional source for the embryo in the developing oocyte. Using specific receptors, they enter the oocytes *via* endocytosis [7] in areas covered by a specific protein called clathrin [8], which is usually located at the base of the oocyte microvilli. After entering, vitellogenins separate from the receptors and form vitellins. Chemical structure of vitellins may differ from that of vitellogenins, and vitellins are stored in a crystalline form in the yolk grains [9]. Not only vitellogenins but also fat droplets from the fat body trophocytes are transported to the oocytes during vitellogenesis. Interestingly, vitellogenin synthesis takes place also directly in the follicular cells of the ovaries in some Diptera species (i.e. flies, mosquitoes) but otherwise, the process is similar to that of vitellogenin synthesis in fat body trophocytes [1].

Briefly, vitellogenesis is a complex neurally and hormonally controlled process in which all groups of insect hormones – juvenile hormones, ecdysteroids, and neurohormones – participate [1]. Nevertheless, there are some differences in this process between insect species, and generally we can divide the control mechanism of vitellogenesis into two groups. In the first group, which includes most insect species except for Diptera, juvenile hormone plays the major role in controlling of vitellogenin synthesis in the fat body. Juvenile hormone is an unusual sesquiterpenoid in chemical nature, produced by corpora allata, the endocrine gland located near the brain and representing one of the major hormones of insect development and metamorphosis. It regulates juvenile development by blocking early metamorphosis, but also has an impact on a number of other processes. In the second insect group, represented mostly by Diptera, the primary role is played by ecdysteroids. Ecdysteroids are insect steroid hormones derived from cholesterol. They are predominantly produced in the prothoracic gland, which is located in the prothorax. In juvenile stages, ecdysteroids regulate moulting and metamorphosis, whereas in adults, they affect reproduction (primarily oocyte maturation). Neurohormones are also responsible for controlling vitellogenesis, however, they often only have minor but still important functions to play in this process [10].

Cascade/regulation of vitellogenin synthesis in insect reproduction

First group – most insect species

Vitellogenin synthesis is commonly triggered by the presence of food (or even just the smell of it) or signals associated with mating. These signals are detected by the brain, which then stimulates the corpora allata to produce juvenile hormones *via* the adenotropic neurohormone allatotropin. Juvenile hormone stimulates the production of vitellogenin in fat body trophocytes by altering the expression of the necessary genes [11]. Thus, the synthesis of vitellogenin includes the classical machinery found in secretory proteins and serves as a model of hormone-regulated gene expression. Formed vitellogenin is then transported from the fat body to the developing oocytes. The entry of vitellogenin molecules into oocytes is facilitated by specific receptors activated by juvenile hormone [12]. Thus, only the juvenile hormone-treated oocyte can receive the transported vitellogenin. Furthermore, in most insect species, a special factor produced by mature oocytes ensures that only the mature oocytes accumulate vitellogenin, while in younger oocytes this process is inactive [13]. The termination of vitellogenin synthesis is triggered by factor(s) from the mature oocytes stimulating corpora cardiaca, small endocrine glands near the brain, to produce adipokinetic hormone that directly inhibits vitellogenin production in the fat body [14]. Nevertheless, vitellogenesis is stimulated not only by juvenile hormone as described above, but also by gonadotropic hormones such as ovary maturing parsin and egg development neurohormone. Nonetheless, the mechanism by which ovary maturing parsin induces the expression of the vitellogenin gene is not fully understood. Vitellogenesis, on the other hand, can also be inhibited, for instance by oostatic hormones (modified according to [1]).

Second group – Diptera

Ecdysteroids, as previously stated, play

an important role in controlling the process of vitellogenesis especially in Diptera [15,16]. However, because prothoracic glands are absent in adults, ecdysteroid production is provided directly by ovariole follicular cells. At first, the filling of the gut with food stimulates production of a gonadotropic neurohormone called egg development neurohormone, which is released from brain neurosecretory cells and which stimulates ecdysteroid production in follicular (ovary) cells. Curiously, the ability to create ecdysteroids only occurs in ovary cells that have been pre-treated with juvenile hormone [17]. Thus, the juvenile hormone is also used in this process of vitellogenesis control, but in contrast to the first group it does not control the process's gene expression. Ecdysteroids, which regulate the synthesis of vitellogenins in trophocytes, play this role. In Diptera and some other insect species such as the lady beetle, *Coccinella septempunctata* [18] and the Colorado potato beetle, *Leptinotarsa decemlineata* [19], vitellogenins are also synthesized in ovary follicular cells [20]. The remaining steps in vitellogenesis control are similar to the process described above (modified according to [1]).

Role of vitellogenins in defense reactions against pathogens and toxins

It is obvious that insect vitellogenins are usually found in sexually mature females. Yet, a small amount of vitellogenins can be found in some insect species' males [21]. For instance, vitellogenins were found in the males of *Pyrrhocoris apterus* [22,23], *Apis mellifera* [24] or *Bombus terestris* [25], among other species. Thus, the question of what role vitellogenins play there arose. Recent research suggested that vitellogenins in these cases are important for regulating immunity, wound healing, life duration, the caste differentiation process in social insects, and several other aspects of insect biology in addition to reproduction [26-30]. As a result, vitellogenins seem to play a more diverse role than their name would imply.

Anti-nematodal activity

There is a diverse range of nematodes acting as entomopathogens in nature. To infect insect hosts, the nematode larvae typically use anal and oral apertures or spiracles. During maturation inside the host body, a variety of venoms and toxins is produced by nematodes themselves, or by their symbiotic bacteria [31,32]. Primarily, these toxins from nematobacterial infections shield nematodes from their insect hosts' immune systems, later contribute to the host death and enable using the host organs as a source of nutrients for growth and development of the nematodes [33-36].

The involvement of vitellogenin in the defense response was affirmed in *P. apterus* infected by the entomopathogenic nematode *Steinernema carpocapsae* [23]. Interestingly, a large increase in vitellogenin transcript level as well as an increase in vitellogenin proteins was observed in the male fat body and haemolymph, respectively (Fig. 1A, C). Surprisingly, the infection had the opposite effect on *P. apterus* females, where a significant decrease in both vitellogenin transcripts and vitellogenin proteins was observed (Fig. 1B, D). Although it is surprising how differently nematode infection affects the male and female bodies, this fact need not be confusing. The different sex-related responses can be explained by great differences in vitellogenin titers between the males and females. As the vitellogenin levels are much higher in females than in males, it appears that the level of vitellogenin needed for effective nematode defense is much lower than that needed for the nutrient supply during the egg development. In order to keep a sufficient amount of energy for more needed functions such as defense reactions, the female stops the process of egg development [37-39] and maintain a low, but adequate, level of vitellogenins for the body's defense against infection. The males must, however, boost vitellogenin production in order to attain this level. A similar trend in a vitellogenin synthesis after the infection was observed also for the Colorado potato beetle *L. decemlineata* treated again with the nematode *S. carpocapsae* [40]. Altogether, these results provide significant support for the conclusions found on *P. apterus* [23]: the Leptinotarsa infection caused a significant increase of vitellogenin transcripts in male fat bodies whilst causing a reduction in female fat bodies (Fig. 2).

Anti-fungal activity

Many pathogenic fungi are insect-specific. These entomopathogenic fungi often begin their infection by physically piercing the host's cuticle and entering the host body. They employ a variety of enzymes in this process, including proteases, lipases, chitinases and chitosanases [41,42]. These enzymes are used by the fungi to break down tissues and organs in the host body, and the resultant material is then used as food for the parasite growth. The creation of toxins, such as

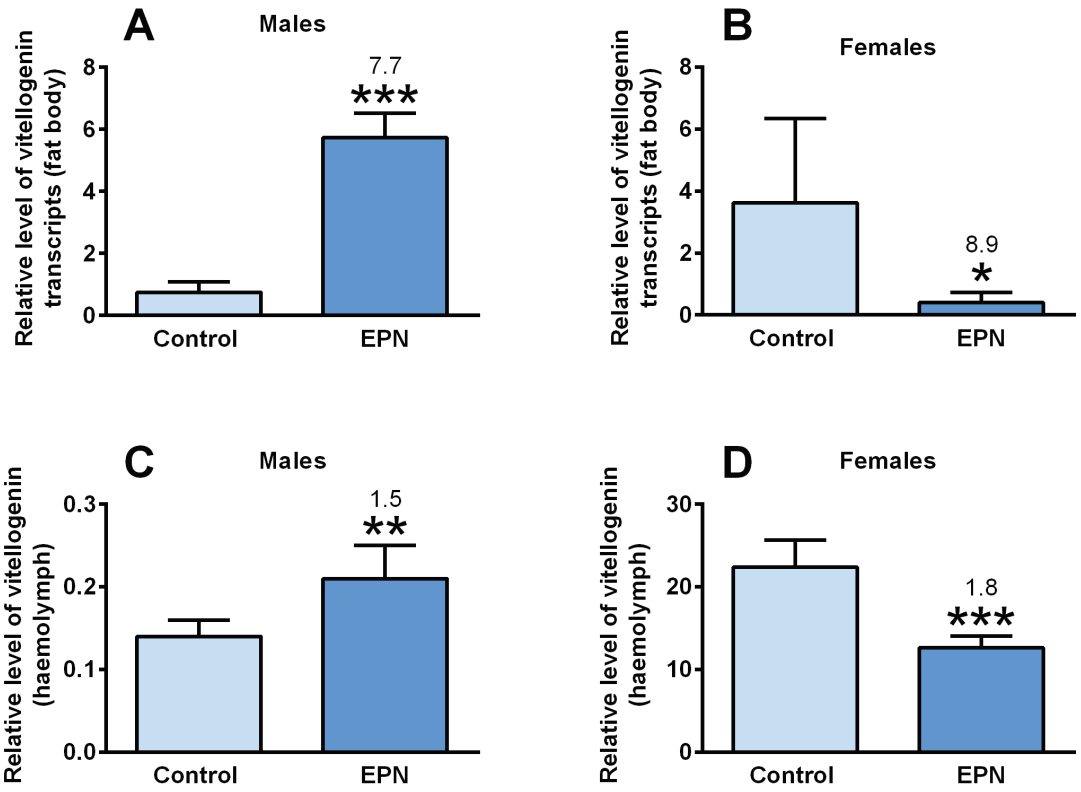

Fig. 1. Effect of the entomopathogenic nematode (EPN) *Steinernema carpocapsae* on vitellogenin transcription in male (**A**) and female (**B**) fat bodies, and on vitellogenin levels in male (**C**) and female (**D**) haemolymph of the firebug *Pyrrhocoris apterus*. Statistically significant difference between infected and control firebugs at 0.1 %, 1 % and 5 % levels evaluated by Student's *t*-test are indicated by ***, ** and *, respectively. The numbers above the bars represent fold-difference of the vitellogenin levels between the infected groups and corresponding controls. Modified according to [23].

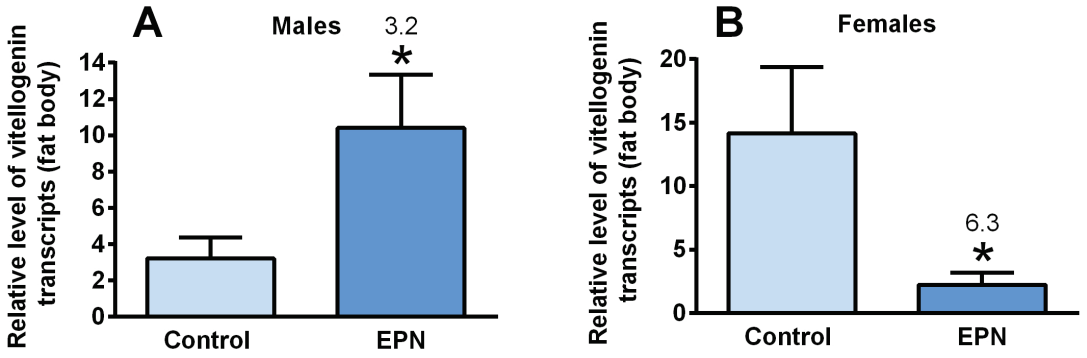

Fig. 2. Effect of the entomopathogenic nematode (EPN) *Steinernema carpocapsae* on vitellogenin transcription in male (**A**) and female (**B**) fat bodies of the Colorado potato beetle *Leptinotarsa decemlineata*. Statistically significant difference between infected and control beetles at 5 % levels evaluated by Student's *t*-test are indicated by *. The numbers above the bars represent fold-difference of the vitellogenin levels between the infected groups and corresponding controls. Modified according to [40].

beauvericin produced by *Isaria fumosorosea*, frequently helps to speed up the process of killing infected cells [43]. It has been found that defense mechanisms against the fungal activity include also vitellogenins. According to research by BenVau and Nieh [44], worker honeybee vitellogenin expression was markedly upregulated in honeybee larvae exposed to *Nosema ceranae* microsporidium spores, and Sinpoo *et al.* [45] found that vitellogenins interact with the cell wall of the entomopathogenic fungus *Beauveria bassiana* to cause membrane disruption and permeabilization.

Besides, it seems, the *I. fumosorosea* infection in *P. apterus* males causes a significant increase of vitellogenin in transcription level in the fat body, while no effect become evident in protein vitellogenin levels in haemolymph (Fig. 3A, C) [23]. Remarkably different

appears to be the response in *P. apterus* females, where a significant decrease of vitellogenin transcript levels were observed in both fat body and haemolymph (Fig. 3B, D). The differences in the reaction between the males and females could be explained by the trade-off between activating defense reactions and sawing energy sources as discussed in chapter Anti-nematodal activity. Nonetheless, why the increase in vitellogenin transcripts is not accompanied simultaneously with vitellogenin proteins in the male body is not satisfactorily explained at this moment (Fig. 3A, C).

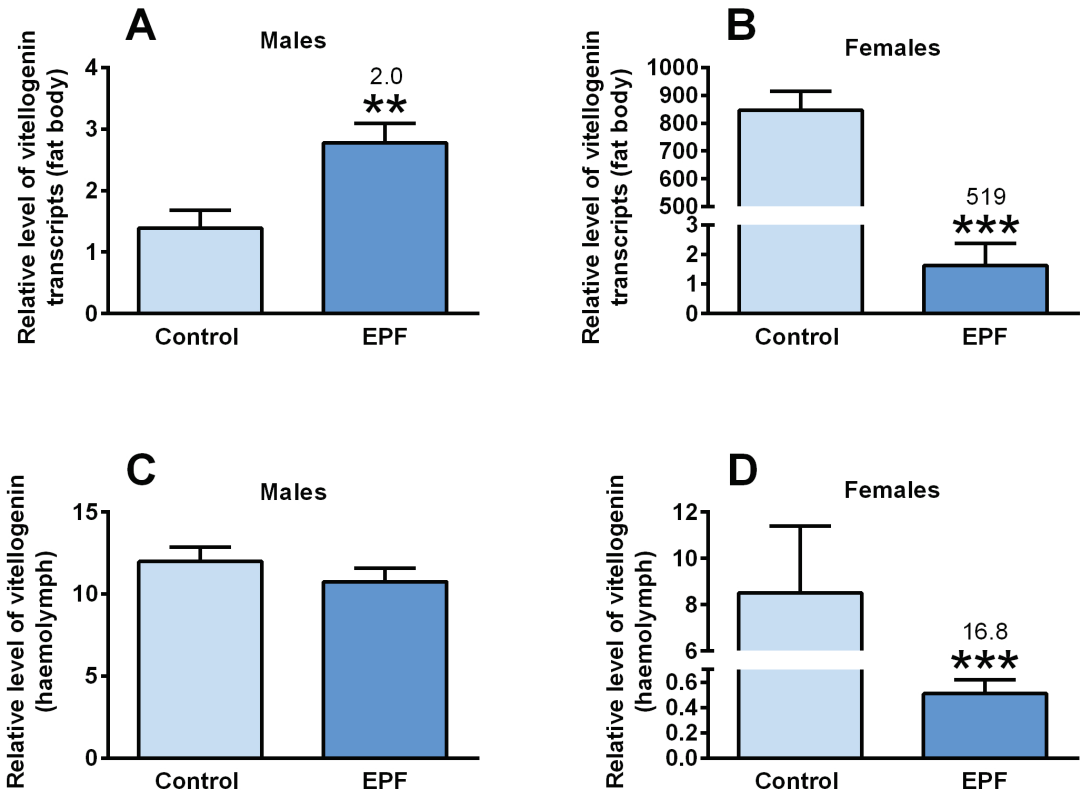

Fig. 3. Effect of the entomopathogenic fungus (EPF) *Isaria fumosorosea* on vitellogenin transcription in male (**A**) and female (**B**) fat bodies, and on vitellogenin levels in male (**C**) and female (**D**) haemolymph of the firebug *Pyrrhocoris apterus*. Statistically significant difference between infected and control firebugs at 0.1 % and 1 % levels evaluated by Student's *t*-test are indicated by *** and **, respectively. The numbers above the bars represent fold-difference of the vitellogenin levels between the infected groups and corresponding controls. Modified according to [23].

Anti-bacterial activity

Vitellogenins are involved also in the defense mechanisms against a variety of entomopathogenic bacteria. The basis of the defense is the vitellogenin adherence to the surface of the bacterium and severe damage to its cell wall, leading to the membrane and membrane permeability disruptions. In this way, vitellogenins effectively combat a gram positive bacterium *Bacillus thuringiensis* and gram negative bacterium *Escherichia coli* in *Apis cerana* [30]. High effectivity was also proved against *Paenibacillus larvae*, a gram-positive bacterium causing the American foulbrood disease, the deadliest bee brood disease in the world [28,46]. The antibacterial effect of vitellogenins was manifested also in the case of the infection of the silkworm *Bombyx mori* by the gram positive bacterium *Bacillus subtilis* and the gram negative bacterium *E. coli* [27]. Noteworthy is also role of vitellogenins as the growth suppressors of the entomotoxic bacteria *Xenorhabdus spp*. isolated from the nematode *S. carpocapsae* [23]. Moreover, vitellogenins might also occur in the gut, where they exhibit antibacterial properties and shield epithelial cells from their own immune reactions [47].

It is interesting to note that vitellogenins appear to cause anti-bacterial trans-generational immune priming in honeybee queens, as they increase immunity in the offspring *via* delivering pathogen-associated pattern molecules into the developing oocytes [28,46,48,49]. It is unclear whether vitellogenins also play a role in anti-viral immune priming;

nonetheless, this possibility is admitted in the work of Lang *et al.* [50]. This eventuality is further strengthened by the fact that vitellogenins bind viruses [51-52].

Anti-bee venom activity

Bee venom is a complex mixture of toxins that frequently act in mutual cascades [53,54]. The primary component of bee venom is melittin, a low-molecular peptide with a variety of biological, pharmacological, and toxicological properties [55,56]. The ability of melittin to bind to the phospholipid layer of the cell membranes and create pores in the membranes is the most important characteristics of its toxic activity. Melittin also activates other enzymes that contributes to the final toxic effect such as, most significantly phospholipases, which cleave phospholipids in cell membranes causing a further membrane perforation and increasing their permeability, and resulting in the leakage of the cell contents and the cell's total destruction. Another contributing enzyme is hyaluronidase. Its main role consists in splitting intercellular junctions and creating fissures between the tissue cells. Therefore, other toxins of the venom can penetrate more easily through the gaps, reaching deeper into the tissues and blood vessels, which accelerates the intoxication. Furthermore, the fragments of substances produced by hyaluronidase cleavage have a significant pro-inflammatory and immunostimulatory effects, increasing the overall impact of bee venom [53,57]. There is also a number of low molecular weight substances in the venom (i.e. salts and amino acids) among which the exceptional role is played by biogenic amines. Histamine, the most important of them, is found not only in the venom, but is also released by injured tissue of the victim in the place of sting. Histamine is a major inducer of inflammatory and immune responses [58]. Other amines, such as dopamine and noradrenaline are responsible for increasing heart activity, resulting in faster venom circulation and distribution throughout the body [54]. Serotonin, in turn, causes an increase in tissue sensitivity, which contributes to pain at the injection site. The bee venom's mechanism of action is non-specific, therefore, it ought to be effective against a wide range of animals, from insects to humans.

Interestingly, the bee venom injection boost a significant drop of a vitellogenin level in males and females of the American cockroach *Periplaneta americana* (Fig. 4) [59]. This pattern completely differs from those observed after the application of entomopathogenic nematodes and fungi (details in chapters Anti-nematodal activity and Anti-fungal activity). Thus, a distinct mechanism might appear to be at play, although its specifics are unknown. Presumably, the insect body response to toxicity may be toxin- and/or species-specific.

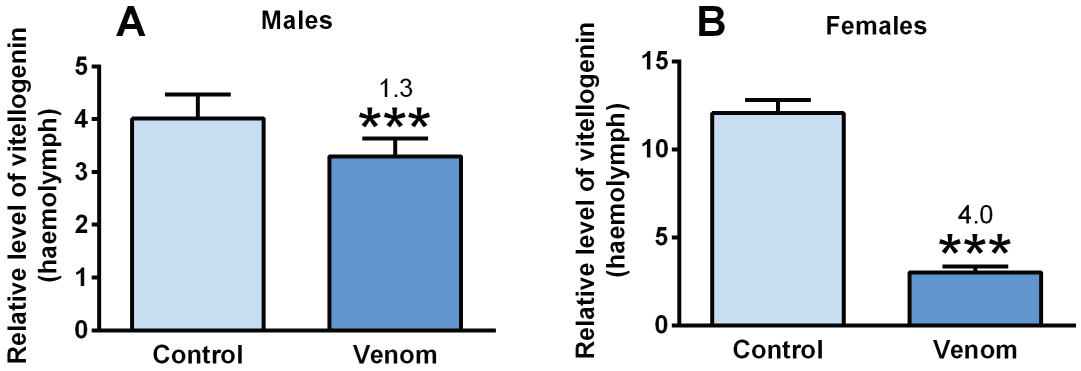

Fig. 4. Effect of the honeybee *Apis mellifera* venom on vitellogenin level in male (**A**) and female (**B**) haemolymph of the American cockroach *Periplaneta americana*. Statistically significant difference between poisoned and control cockroaches at 0.1 % levels evaluated by Student's *t*-test are indicated by ***. The numbers above the bars represent fold-difference of the vitellogenin levels between the poisoned groups and corresponding controls. Modified according to [58].

Role of vitellogenin in honey bee venom

As previously stated (chapter Anti-bee venom activity), honey bee venom is a complex cocktail of various toxins which, surprisingly, also contains a trace of vitellogenin [60]. The exact function of vitellogenin in honey bee venom is not reliable known, however, Blank *et al.* [60] proposed that vitellogenin might expanse allergic reactions in the victim's body. Recently Kodrík *et al.* [61] revealed that vitellogenin levels in honeybee venom fluctuate throughout the year with a peak in October when the bees are still relatively active.

Surprisingly, this is not correlated to the vitellogenin peak in honeybee haemolymph, which occurs in December/January [61-63] and which is expected to play a role in preventing winter stress, particularly oxidative stress (chapter Role of vitellogenins in anti-oxidative stress reactions). As a result, the functional relationship between vitellogenin levels in venom and haemolymph is unknown, and more research is needed to fully explain the role(s) of vitellogenins in bee venom.

It is mostly unknown whether vitellogenins are present in other hymenopteran venoms. Nonetheless, vitellogenin precursors or vitellogenin-like proteins appear to be present in common [64] and some solitary wasps [65].

Role of vitellogenins in anti-oxidative stress reactions

Reactive oxygen species (ROS), which are unavoidable by products of aerobic metabolism, accumulate in the body and lead to oxidative stress [66]. The anti-oxidative responses have evolved into a variety of defense mechanisms involving enzymatic and non-enzymatic strategies [67]. In insects, these processes are frequently regulated by adipokinetic hormones [36,68,69]. Given that vitellogenins have been demonstrated to elicit a protection against oxidative stressors like paraquat or hydrogen peroxide, where they work as a potent ROS scavenger, they play a significant part in anti-oxidative processes [70]. The anti-oxidative effect of insect vitellogenin has been demonstrated in both insect and mammalian cells [30]. According to several studies [26,70,71], the anti-oxidative function of vitellogenin is thought to be the primary cause of the extended lifespan of honeybee queens and winter bees (for the detailed role of vitellogenins in longevity see Role of vitellogenin in longevity). The specific mechanisms by which vitellogenins reduce oxidative stress in insect bodies are unclear, though.

Role of vitellogenin in longevity

The fact that the endocrine system led by gonadal hormones contributes to lifespan regulation was first proposed by Brown-Séguard, one of the fathers of endocrinology, in the late 1800s and confirmed in the 20th century by showing that removing of gonads and single gene mutations in the highly conserved insulin/IGF-1 signaling pathway lengthen life in various animal species (reviewed in [72]). A trade-off between energy expenditure on somatic maintenance or reproductive performance has also been proposed as a common phenomenon involved in the regulation of lifespan, in order to explain life history strategies, and numerous studies on various animal species support the idea that caloric restriction can function as a common lifespan stimulating factor [73-76].

In the regulation of adult lifespan of insects, ecdysteroids and juvenile hormones are involved as aging promoting factors [21,77]. The long-lived insulin receptor Drosophila mutants display reduced levels of both hormones, and the impairment of their biosynthesis leads to lifespan extension [78-80]. As mentioned above (more details in chapter Role of vitellogenins in reproduction), in most insects, juvenile hormone triggers reproductive process resulting in vitellogenin production [78,80-82], but in honeybees, juvenile hormone and vitellogenin levels are negatively correlated and have a mutually suppressive effect [71,83,84]. Young bees consume a diet high in protein, which stimulates vitellogenin synthesis, resulting in high vitellogenin levels and low levels of juvenile hormone. Further, the expression of vitellogenin declines with age, and low vitellogenin levels cause a rise in juvenile hormone levels triggering foraging activity. Foraging is associated with a diet high in carbohydrates but low in proteins, but also it is linked to higher insulin/IGF-1 signaling levels and aging rate [85-87]. Additionally, vitellogenin – juvenile hormone regulatory module controls changes in carbohydrate metabolism applied during the transition into foraging [88].

High vitellogenin levels along with low juvenile hormone levels are found in the long-lived winter generation of workers, while the short-lived summer workers show the lowest vitellogenin levels and highest levels of juvenile hormone. As reduced photoperiod is believed to stimulate the formation of the fat body mass, photoperiod shortening during a year leads to the increased levels of vitellogenin, which decrease juvenile hormone levels and potentially lead to a prolong lifespan and formation of the long-lived winter worker generation [63,89-91].

Queens of eusocial insects typically contradicts the usual trade-off between fecundity and lifespan as are both extremely long-lived and fecund (reviewed in [92]). Honeybee queens live 100 times longer and have lower insulin/IGF-1 signaling level compared to workers, but they also have lower levels of juvenile hormone and significantly higher levels of vitellogenin [86,87,93].

Together, vitellogenin appears to play a crucial role in regulating social behavior and lifespan in honeybees. Additionally, because vitellogenin performs a critical antioxidant role (chapter Role of vitellogenins in anti-oxidative stress reactions), it is thought that its antioxidant properties may also contribute to the differences in honeybee lifespan [86,94].

Conclusions

Without any disputes, vitellogenins are not only passive reservoirs of energy nutrition for the developing embryo in insect eggs, but also have a wide range of bioactive roles, particularly in the defense of insect organisms against diseases, infections, intoxication, and/or oxidative stress, and play role in lifespan regulation (Fig. 5). Although most of mechanisms of their action are unclear it seems they may vary depending on the species, venom composition, specificity of infection, or general stressful circumstances. Understanding the details of vitellogenin protective mechanisms and identifying weaknesses in the mode of their action could have practical implications for pest control.

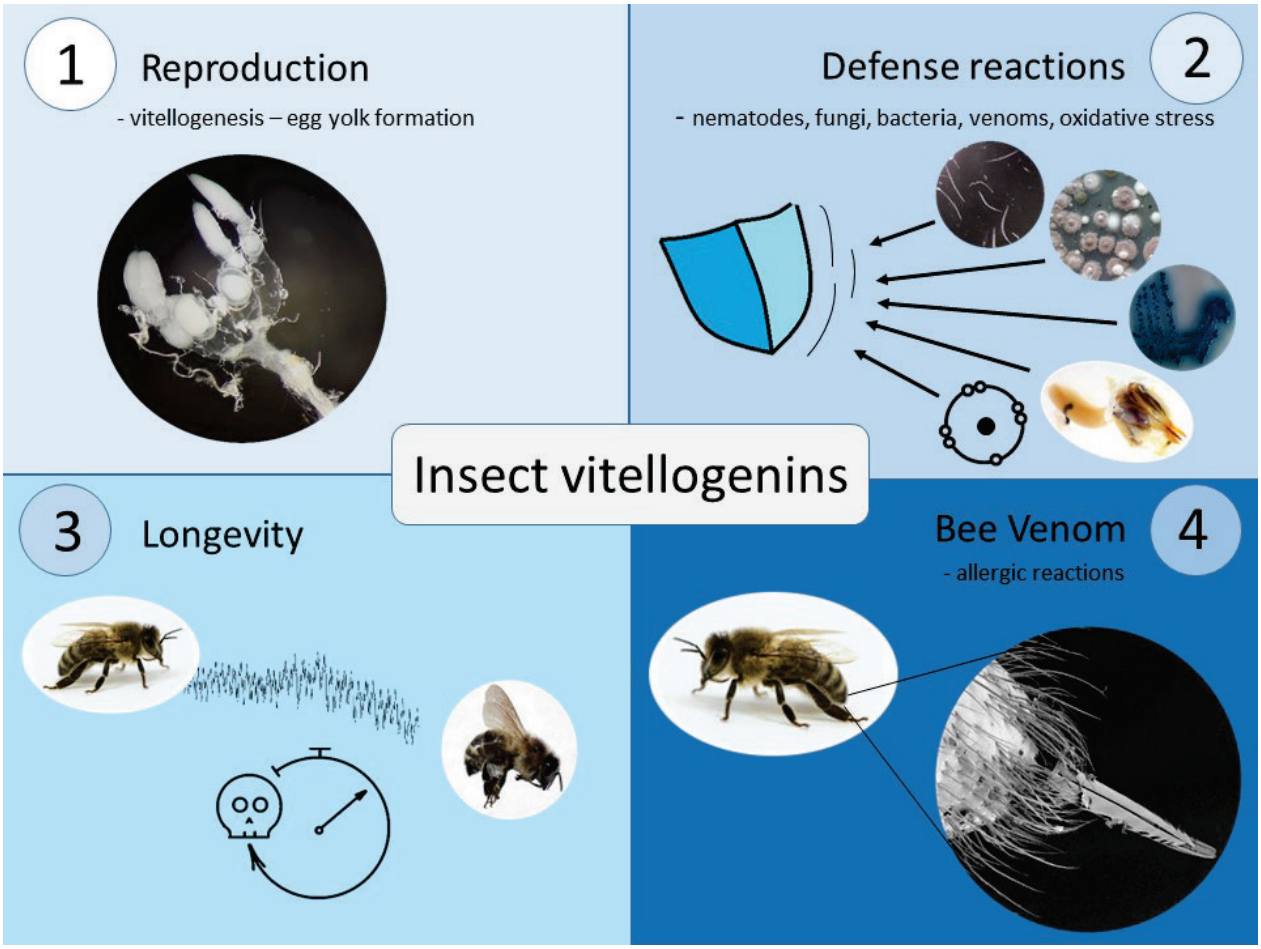

Fig. 5. Diagram illustrating roles of vitellogenins in the insect body: 1 – primary role of vitellogenins in insect (female) reproductive vitellogenesis. 2 – role of vitellogenins in defense reactions against pathogens, venoms and toxins. 3 – role of vitellogenins in the (bee) lifespan regulation. 4 – role of vitellogenins in the bee venom. Figures kindly provided by D. Hlávková, J. Konopická, J. Nermuť, F. Weyda and K. Sláma.

Conflict of Interest

There is no conflict of interest.

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