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Temporal variation of vitellogenin synthesis in *Ectatomma tuberculatum* (Formicidae: Ectatomminae) workers

Dihego Oliveira Azevedo [a], José Cola Zanuncio [a], Jacques Hubert Charles Delabie [b], José Eduardo Serrão [c,*]

[a] Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG, Brazil
[b] Laboratório de Mirmecologia, Convênio CEPLAC-UESC, Itabuna, BA, Brazil
[c] Departamento de Biologia Geral, Universidade Federal de Viçosa, Av. P. H. Rolfs s/n, 36570-000 Viçosa, MG, Brazil

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ABSTRACT

Workers of the ant species *Ectatomma tuberculatum* (Ectatomminae) have active ovaries and lay eggs that are eaten by the queen and larvae (trophic eggs). Vitellogenins are the main proteins found in the eggs of insects and are a source of nutrients. The aim of this study was to characterize the period of vitellogenin production in workers of *E. tuberculatum*. The vitellogenin was identified from queen and worker eggs by SDS-PAGE. Anti-vitellogenin antibodies were obtained and used to detect this protein in the fat body and haemolymph of workers at different ages. Vitellogenin from *E. tuberculatum* consists of two polypeptides of 31 and 156 kDa. In the eggs of queens, the 156 kDa polypeptide is cleaved into two subunits of 36 and 123 kDa. The analysis of the haemolymph of workers showed that the secretion of vitellogenin varies with age. The secretion is initiated around the fifth day after emergence, with peak production from days 20 to 60, and stops around day 100. The variation in production is related to the different activities performed by the workers within the colony, suggesting that vitellogenin may have an important role in maintaining age polyethism.

1. Introduction

Vitellogenin is the precursor of vitellin, a phospholipoglycoprotein that constitutes the major fraction of the egg yolk proteins in insects and is the main source of nutrients for the embryo (Raikhel and Dhadialla, 1992; Tufail and Takeda, 2008). In insects, the amino acid sequence of vitellogenins is conserved at many sites (Chen et al., 1997; Tufail and Takeda, 2008), although the number of genes that encode them varies in different species. In hemimetabolous insects, one gene is present in *Blattella germanica* (Blattaria) (Comas et al., 2000) and two genes in *Leucophaea maderae* (Blattaria) (Tufail et al., 2007). For holometabola insects, five genes were identified in *Aedes aegypti* (Diptera) (Chen et al., 1994), one in both *Bombyx mori* (Lepidoptera) (Yano et al., 1994) and *Apis mellifera* (Hymenoptera) (Piulachs et al., 2003), and three in *Solenopsis invicta* (Hymenoptera) (Tufail and Takeda, 2008).

Vitellogenin is mainly synthesized in the fat body of females, where single or multiple polypeptides undergo modifications such as glycosylation, lipidification, phosphorylation, sulfation, and proteolytic cleavage (Tufail and Takeda, 2008). They are then released into the haemolymph as oligomeric proteins with molecular weights ranging from 300 to 600 kDa (Tufail and Takeda, 2008; Wheeler et al., 1999). These protein aggregates are then transferred to oocytes via receptor-mediated endocytosis and stored in the form of crystals, at which time they are termed vitellins (Giorgi et al., 1999; Raikhel and Dhadialla, 1992).

In social insects, the production of vitellogenins is not exclusive to queens, the reproductive females, but also occurs in the non- or subfertile worker castes (Engels, 1974; Guidugli et al., 2005; Seehuus et al., 2006), and in the honey bee it was even found in males (Piulachs et al., 2003; Trenscek et al., 1989). Workers of the stingless bee *Frieseomelitta varia* are sterile but produce vitellogenin constitutively throughout their life (Dallacqua et al., 2007). In several species of ants, workers may activate ovaries and produce unfertilized eggs, termed trophic eggs, that are used to feed the brood and the reproductive castes (Dietemann and Peeters, 2000; Fénéron and Billen, 1996; Gobin et al., 1998; Hölldobler and Wilson, 1990). The production of vitellogenin by the non-reproductive castes suggests that it has functions in addition to supplying nutrients to the embryo, which have been better characterized in bees (Amdam et al., 2003).

In *A. mellifera* workers, variation in their production of vitellogenin is related to their permanence inside the colony and the onset of foraging flights (Marco Antônio et al., 2008; Nelson et al., 2007). Production of vitellogenin also increases the longevity of queens when compared to workers by reducing their rate of aging through resistance to oxidative stress (Corona et al., 2007; Seehuus et al., 2006). Vitellogenins have important functions in

* Corresponding author. Tel.: +55 31 38991301; fax: +55 31 38992549.
E-mail address: jeserrao@ufv.br (J.E. Serrão).

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somatic maintenance and in the immune system of bees (Amdam et al., 2004; Seehuus et al., 2006), and are part of the insulin/insulin-like signaling pathway, which regulates growth, aging, and reproduction in vertebrates and invertebrates (Corona et al., 2007).

The ant species *Ectatomma tuberculatum* (Ectatomminae) forms colonies of up to 400 workers and one or more queens (Hora et al., 2005). The workers have the same size and are morphologically different from queens, and perform different tasks in the colony according to their age (Fénéron and Billen, 1996; Fénéron et al., 1996). The workers also have active ovaries that produce trophic eggs (Fénéron and Billen, 1996; Hora et al., 2007) and the development of their ovaries is related to their age (Fénéron et al., 1996). Therefore, the production of vitellogenin is related to nourishing colony members and possibly to the different activities performed by workers. In this work we test the hypothesis that the period of vitellogenin production is linked to intranidal activities in age polyetism of *E. tuberculatum* workers. We find that vitellogenin is produced when workers are inside the nest acting in brood care and ceasing when workers are in activities out of the nest, suggesting that this protein can be used as a nutrient supplement since the eggs produced by workers are trophic eggs that are used for queen and brood feeding.

2. Materials and methods

2.1. Insects

Five *E. tuberculatum* colonies were provided by the Laboratory of Myrmecology at the Cocoa Research Centre (CEPLAC) in Itabuna, Brazil. The ants were kept in artificial colonies built in plastic cages (18 cm × 25 cm) and filled with plaster. The colonies were connected by tubes to other cages (10 cm × 10 cm) cm) without plaster that were used as foraging areas. All colonies were polygynous, containing two to five queens and more than 30 workers in addition to the brood. The colonies were maintained at 26 ± 2 °C and fed every two days with *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae, honey, and water *ad libitum*. In order to obtain ants with known ages, newly emerged workers were marked with an enamel paint dot on the thorax and returned to their colonies.

2.2. Haemolymph extraction

Haemolymph samples were collected from mated and active egg laying queens of unknown age and workers 2, 5, 10, 15, 20, 30, 60, and 100 days old. The workers were cryo-anesthetized (0 °C) and decapitated, while queens had an incision made in their thorax with a sterile entomological pin. Between 0.5 and 0.75 μL of haemolymph was collected from each ant by microcapillary. The queens were put back in their colonies of origin after extraction. The collected haemolymph was added to a tube containing 20 μL of Tris–HCl (0.05 M, pH 7.2) with 15% (v/v) of protease inhibitor cocktail [4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), E-64, bestatin, leupeptin, aprotinin, and sodium EDTA (Sigma-Aldrich)].

2.3. Vitellogenin samples

E. tuberculatum vitellogenin and/or vitellin samples were obtained from newly laid queen eggs and mature oocytes dissected from workers' ovaries.

Eggs and oocytes were macerated in 0.05 M Tris–HCl buffer, pH 7.2, containing 15% (v/v) of protease inhibitor cocktail (Sigma). The extracts were centrifuged at 9300 × *g* for 10 min and the supernatant was collected. The soluble proteins present in the extracts were quantified according to Bradford (1976) using bovine serum albumin as a standard.

2.4. SDS-PAGE

The haemolymph samples and egg extracts from the queens and workers were subjected to electrophoresis on a 12% polyacrylamide gel containing sodium dodecyl sulfate (SDS-PAGE) (Laemmli, 1970) in order to assess the protein profiles. The samples were diluted to a ratio of 1:2 (v/v) in sample buffer [20% (v/v) of 10% SDS, 12.5% (v/v) 0.5 M Tris–HCl pH 6.8, 25% (v/v) glycerol, 0.01% (w/v) bromophenol blue, 5% (v/v) β-mercaptoetanol], boiled for 4 min, and run on the gel. We used 5 μg of protein from egg extracts and 5 μL of diluted haemolymph samples. The gel was stained with a Coomassie blue solution (2% blue Coomassie G250, 10% acetic acid, 47.5% ethanol). The molecular weights of the proteins were determined with a standard curve based on a linear regression between the log of molecular weight of standard proteins (Promega™ Broad Range Protein Molecular Weight Marker) and their rf-values.

2.5. Antibody production

The two major vitellin proteins identified in queen eggs on SDS-PAGE were isolated and used in the production of anti-vitellogenin antibodies.

Each putative vitellogenin protein was used as antigen for immunization of three rabbits up to three months old. In the initial immunization a total of 1 mg of protein mixed with Freund's complete adjuvant (v/v) was injected subcutaneously. The second and third booster immunizations were performed 30 and 60 days after the first, each of them using a total of 0.25 mg of protein mixed with incomplete Freund's adjuvant (v/v). The rabbits were bled 30 days after the third immunization and the serum containing the antibodies was obtained and stored at −20 °C.

2.6. Western blotting

Haemolymph samples and egg extracts were subjected to SDS-PAGE as described above. The gel was incubated for 20 min in transfer buffer (0.58% Tris base, 0.28% glycine, 0.037% SDS, 20% methanol), followed by transfer of the proteins to a 0.2 μm pore size nitrocellulose membrane (Sigma–Aldrich, cat. N7892-5EA). The membrane was incubated overnight at room temperature with 5% non-fat dried milk in 0.1 M TBS buffer, pH 8.0, containing 0.1% Tween-20 (TBST), washed in TBST, and incubated for 2 h at room temperature with the anti-vitellogenin antibodies diluted 1:1500 in TBST with 2.5% non-fat dried milk. The negative control was done by incubating samples of queen egg extracts with rabbit pre-immune serum diluted 1% in TBST with 2.5% non-fat dried milk. The membrane was washed in TBST, incubated with anti-rabbit IgG conjugated to horseradish peroxidase (Sigma-Aldrich) diluted 1:4000 in TBST with 2.5% non-fat dried milk for 2 h at room temperature, washed, and revealed with DAB/H_2O_2 solution (0.1% 3-3′-diaminobenzidine in 50 mM Tris–HCl, pH 7.6, 2.5% of 0.3% nickel chloride in H_2O, 0.1% H_2O_2).

2.7. Immunohistochemistry

Samples of fat body from workers aged 30 days were dissected, fixed in Zamboni's solution (Stefanini et al., 1967) for 30 min, dehydrated in alcohol, and embedded in LR White resin. Sections 5 μm thick were treated with 1% phenylhydrazine in 0.1 M PBS, pH 7.4, for 30 min, washed in PBS, and incubated with 2% (v/v) normal rabbit serum in PBS for 20 min. The sections were then incubated with anti-vitellogenin antibody diluted 1:500 in PBS for 2 h at 37 °C, washed in PBS and incubated with anti-rabbit IgG conjugated to horseradish peroxidase (Sigma-Aldrich) diluted 1:1000 in PBS for 2 h at 37 °C. The sections were washed in PBS and

0.05 M Tris–HCl, pH 7.6, revealed with DAB/H_2O_2, and counter-stained with hematoxyline.

2.8. Immunofluorescence

Samples of fat body and oocytes were obtained by dissection of workers aged 30 days. The samples were transferred to Zamboni's fixative solution (Stefanini et al., 1967) for 30 min and washed with 0.1 M PBS, pH 7.4, with 0.1% Tween-20 (PBST). They were then incubated in a solution of 1.5% bovine serum albumin in PBST for 10 min at 37 °C, washed in PBST, and incubated in 2% normal rabbit serum in PBST for 20 min at 37 °C. The samples were washed again in PBST and incubated overnight at 4 °C with anti-vitellogenin antibody diluted 1:500 in PBST. Then, the samples were incubated for 2 h at 37 °C with anti-rabbit IgG conjugated with FITC (Sigma-Aldrich) diluted 1:100 in PBST. The samples were mounted on microscope slides in a 50% sucrose solution and viewed under a fluorescent microscope (Olympus BX-50) with an excitation filter of 495–530 nm. The negative control was performed by omitting the anti-vitellogenin antibody.

2.9. Native PAGE

Egg extracts from queens and workers and haemolymph samples from workers with 30 days of age were subjected to discontinuous native PAGE (Davis, 1964) in order to compare the native vitellins and vitellogenins. Samples containing 2.5–5 μg of proteins were diluted 1:2 (v/v) in sample buffer [12.5% (v/v) 0.5 M Tris–HCl pH 6.8, 30% (v/v) glycerol, 0.01% (w/v) bromophenol blue] and applied onto a 7% polyacrylamide gel. After the electrophoresis the gel was prefixed overnight with methanol acetic solution [10% (v/v) methanol, 10% (v/v) glacial acetic acid], fixed for 1 h with 12.5% formaldehyde solution and stained with ammoniacal silver nitrate solution [0.15% (w/v) silver nitrate, 0.05% (w/v) sodium hydroxide and 2.5% (v/v) ammonium hydroxide].

3. Results

SDS-PAGE analysis comparing egg proteins from *E. tuberculatum* queens and workers showed differences between the castes. The eggs of the workers contained two major proteins with molecular weights of 31 and 156 kDa, while the eggs of queens had eight major proteins, four of which (31, 36, 123, and 156 kDa) appeared with greater intensity than the others (81, 86, 96, and 101 kDa) (Fig. 1).

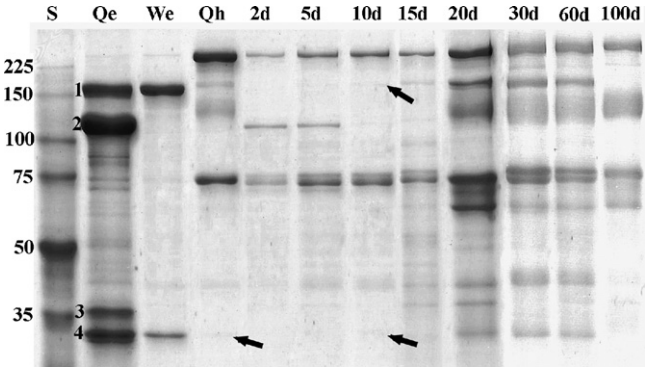

Fig. 1. SDS-PAGE showing the protein profile from eggs and haemolymph of *E. tuberuclatum* queens and workers. The putative vitelins are indicated by the numbers 1 (156 kDa), 2 (123 kDa), 3 (36 kDa) and 4 (31 kDa). The vg1 and vg2 antibodies were made using the proteins 1 and 2. Arrows shows protein weakly stained. S, standart molecular weight followed by their respective values in kDa. Queen egg (Qe). Worker egg (We). Queen haemolymph (Qh). Worker haemolymph with 2 (2d), 5 (5d), 10 (10d), 15 (15d), 20 (20d), 30 (30d), 60 (60d) and 100 (100d) days of age.

Haemolymph samples from workers of different ages displayed different protein patterns. Proteins with MWs of 43, 84, 89, and 195 kDa occurred in samples from workers of all ages (Fig. 1). The haemolymph of workers aged 2 and 5 days had a 120 kDa protein that was not found in workers of other ages. Haemolymph from workers with 10 days of age showed small quantities of the proteins of MWs 31 and 156 kDa present in worker oocytes (Fig. 1). These proteins also appeared in the haemolymph of workers aged 15, 20, 30, and 60 days. From the age of 20 days, all ants expressed the proteins of 38, 71, and 135 kDa. Workers 100 days of age did not show the proteins of 31 and 156 kDa (Fig. 1). In the haemolymph of queens, proteins of 85, 135, 156 and 195 kDa appeared in greater quantity, while the 31 and 43 kDa proteins were slightly detected (Fig. 1).

To verify the presence of vitellogenin in ants of different ages, the two most abundant proteins in eggs of queens (Fig. 1) were isolated and used to immunize rabbits for antibody production. The antibodies obtained to proteins of 123 and 156 kDa were termed vg1 and vg2, respectively.

Immunolocalization tests were performed to provide indirect evidence that the isolated proteins may correspond to vitellogenin. Immunohistochemistry (Fig. 2A–C) and immunofluorescence (Fig. 2D–F) showed positive reactions for antibodies vg1 and vg2 in fat body cells and oocytes. The fat body was characterized by large cells that had a central nucleus and many vacuoles (Fig. 2A–C). The immunostained granules were found around these vacuoles and were clustered at the cell periphery (Fig. 2A and B). In the oocytes, the positive granules were observed throughout the ooplasm (Fig. 2E).

The antibodies vg1 and vg2 were used in Western blot analysis of egg extracts from queens and workers, while the haemolymph samples were analyzed using only the vg2 antibody. The analysis showed that both antibodies reacted positively to the proteins of 123 and 156 kDa and also for smaller unspecific fragmentation products (Fig. 3). Analysis of the haemolymph showed a positive reaction to a protein of 156 kDa in samples from queens and workers aged 5, 10, 15, 20, 30, and 60 days (Fig. 4). An increase in the intensity of the reaction was obtained in samples from workers aged 20 and 30 days. The intensity decreased at 60 days of age but it was higher than at 10 and 15 days of age. Workers aged 2 and 100 days had no positive reactions for the anti-vitellogenin antibody (Fig. 4). Neither the 120 kDa protein found in workers aged 2 and 5 days nor the protein of 135 kDa from samples of workers aged 20–100 days reacted positively to the vg2 antibody (Fig. 4).

The native vitellins from queens and workers were compared and their eggs showed one main protein with the same size (Fig. 5). In the haemolymph from 30 days old workers a similar protein was also identified (Fig. 5).

4. Discussion

In eggs from queens of ant species in the families Myrmicinae, Ponerinae and Ectatomminae the vitellin is formed by two or more proteins (Lewis et al., 2001; Wheeler et al., 1999). Our results show that the eggs of *E. tuberculatum* queens have vitellins that consist of four major proteins, while in the eggs of workers only two of them are the vitellins. In queens, these four proteins join to form an oligomeric protein which in its native form has a molecular weight between 400 and 500 kDa estimated based on data from Wheeler et al. (1999). In the eggs of workers, the 156 and 31 kDa vitellins form an oligomeric protein with the same molecular weight of the native vitellin found in queens.

The two proteins found in the greatest amounts in the egg extracts of *E. tuberculatum* were used for antibody production because the vitellins make up the largest fraction of proteins found in the eggs of insects (Raikhel and Dhadialla, 1992; Tufail and

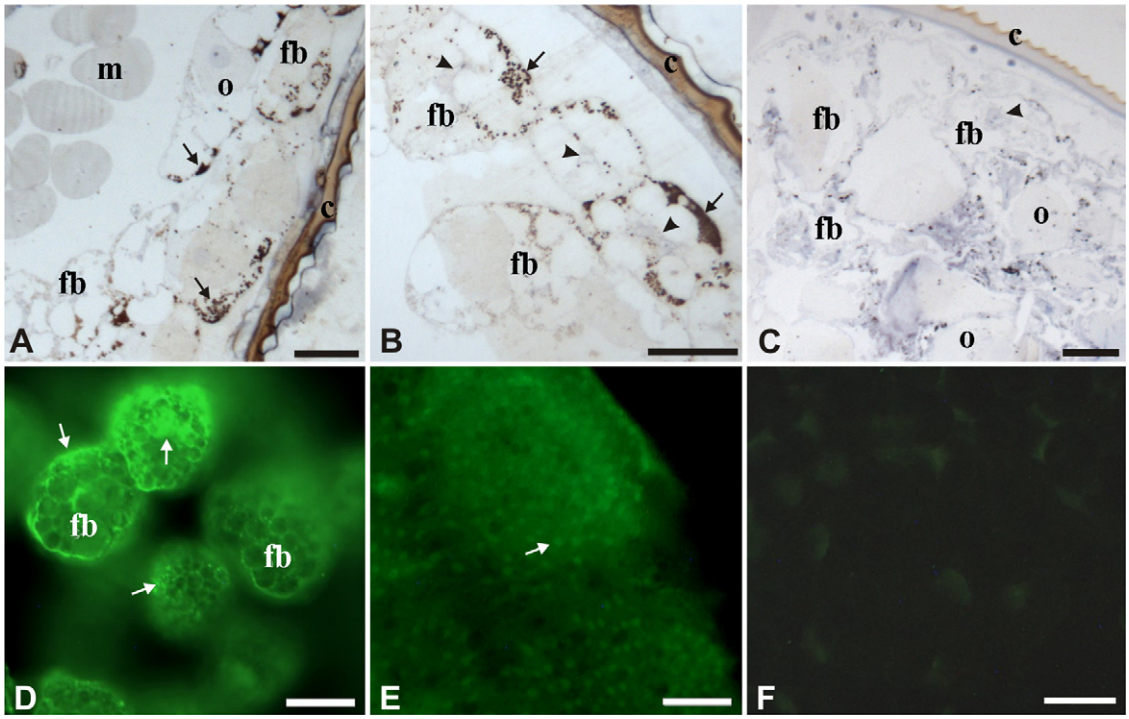

Fig. 2. Vitellogenin in organs of thirty days old *E. tuberculatum* workers. (A and B) Immunohistochemistry in fat body cells showing positive staining granules (arrows). (C) Immunohistochemistry negative control. Tha dark granules are artefacts of the couterstain procedure with hematoxilyn. Bar = 20 μm (light microscope). The vg1 anti-vitellogenin antibody was used in A, while the vg2 antibody was used in (B). Immunofluorescence in fat body cells (D) and oocytes (E). The positive staining is evidenced by the strong greenish, as indicated by arrows. (F) Immunofluorescence negative control in fat body cells. Bars = 50 μm (fluorescence microscope).The vg2 antibody was used in (D) and the vg1 antibody was used in (E). c, Tergite's cuticle; fb, fat body cells; m, muscle cells; o, oenocytes. arrowheads, nuclei of fat body cells.

Takeda, 2008). The immunolocalization tests showed that these proteins occur in fat body cells, the main production site of vitellogenins. Since vitellogenins are the precursors of vitellins (Chapman, 1998), our results confirm that these two proteins are actually vitellin compounds.

Comparing the vitellins of the queen and worker eggs with the vitellogenins from their haemolymph revealed that only the proteins of 31 and 156 kDa were shared, suggesting that the vitellogenin circulating in the haemolymph of *E. tuberculatum* consists of only these two proteins. Also, the presence of a native protein in worker's haemolymph with similar size of the native

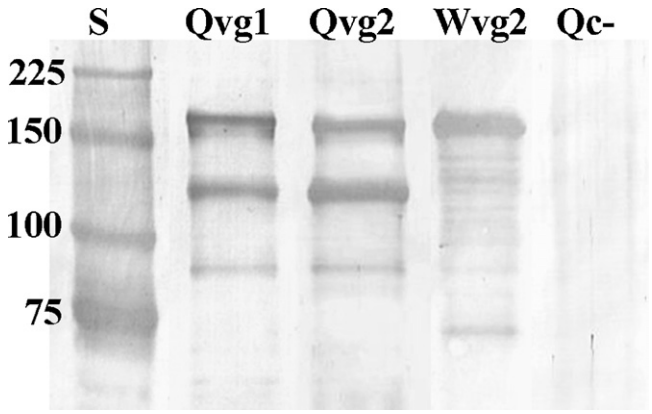

Fig. 3. Western blotting in *E. tuberculatum* queen and worker's eggs with anti-vitellogenin antibodies. The vg1 and vg2 antibodies showed positive reaction with the 123 and 156 kDa proteins. These proteins did not react with the pre-immune serum. Note the unspecific positive reaction showed by minor proteins. S, standart molecular weight protein pattern with respective masses in kDa. Qvg1, queen's egg proteins reacted with vg1 antibody; Qvg2, queen's egg proteins reacted with vg2 antibody; Wvg2, worker's egg proteins stained by vg2 antibody. Qc – queen's egg proteins incubated with rabit pre-immune serum (negative control).

vitellins found in queen and worker eggs indicates that the vitellogenin forms a protein complex in the haemolymph similar to the vitellins found in the eggs. The proteins of 36 and 123 kDa present in the eggs of queens may be products of additional cleavage of the 156 kDa protein, which is supported by the occurrence of cross-reactivity between antibodies vg1 and vg2 to the proteins of 123 and 156 kDa. Moreover, the haemolymph of the queens shows only the proteins of 31 and 156 kDa. In *B. germanica*, vitellogenins of 160 kDa are cleaved into subunits of 50 and 95 kDa after internalization in the oocyte (Wojchowski et al., 1986).

The difference in vitellin processing found between queen and worker eggs of *E. tuberculatum* may be due the absence of active proteases in worker's eggs or the absence of their activation inside the trophic eggs, which have no embryonic development (Giorgi et al., 1999; Khila and Abouheif, 2008). In the course of embryonic development, the vitellin peptides undergo specific cleavages inside the oocyte resulting in new smaller peptides. These cleavages occur due proteases associated with the yolk granules, that may be synthesized inside the oocyte or extraovarially, and activated during the embryonic development (Giorgi et al., 1999).

The lack of immunoreactivity of the vg2 antibody against the 36 kDa fragment may be due to low immunogenicity of this

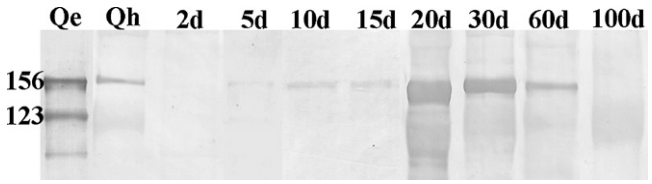

Fig. 4. Western blotting in haemolymph samples of *E. tuberculatum* queen (Qh) and worker with 02 (2d), 05 (5d), 10 (10d), 15 (15d), 20 (20d), 30 (30d), 60 (60d) and 100 (100d) days of age stained with vg2 antibody. Qe, Queen egg proteins showing the 123 and 156 kDa vitellins stained with the vg2 antibody.

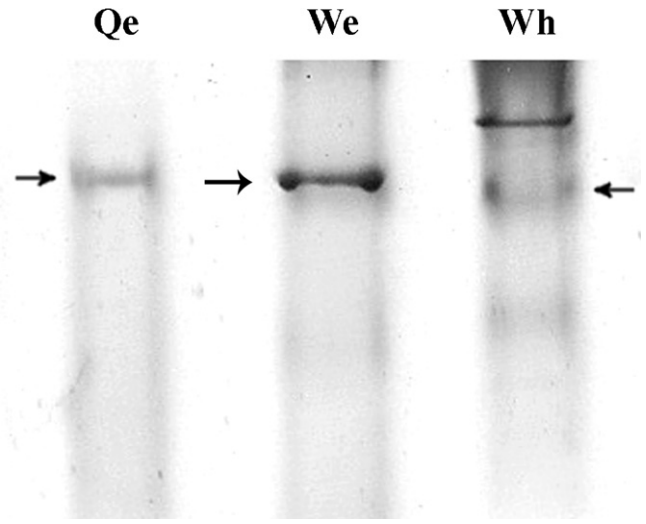

Fig. 5. Native discontinuous PAGE showing the vitellins from *E. tuberculatum* queens (Qe) and workers (We) eggs and the vitellogenin (arrow) in workers haemolymph (Wh). The molecular weight of vitellin in queen eggs was estimated between 400 and 500 kDa (Wheeler et al., 1999).

protein portion or because there is a small fraction of antibodies in the polyclonal serum raised against the 156 kDa protein that bind to the 36 kDa fragment. In *A. mellifera* workers, the 180 kDa full-length vitellogenin is cleaved in two distinct fragments in the fat body, being one small N-terminal of 40 kDa and one large C-terminal of 150 kDa, and the antibodies produced against the 180 kDa vitellogenin fail in recognize the 40 kDa fragment (Havukainen et al., 2011). The 36 kDa protein of *E. tuberculatum* queen eggs was also used as an immunogen, but the antibody obtained was unsatisfactory. The small proteins present in the queen egg extracts that reacted unspecifically with the vg1 and vg2 antibodies may be artefacts of the extraction process.

About some other proteins present in the haemolymph of *E. tuberculatum*, the 195 kDa and 80 kDa may be lipophorin and hexamerin subunits, respectively, like described for some ants (Martínez et al., 2000; Wheeler and Buck, 1995; Wheeler and Martínez, 1995). The lipophorins are important for lipid transport, while the hexamerins may have functions in nutrient storage, hormone carriers, immune protection and cuticle formation (Burmester, 1999). The 120 kDa protein found in the haemolymph of *E. tuberculatum* workers with 2 and 5 days of age may be a hexamerin remaining from the pupal stages that is depleted from the haemolymph during the first days of adult lifespan, likely found for a 110 kDa hexamerin in other ants (Wheeler and Buck, 1995).

Our results indicate that the production of vitellogenin in *E. tuberculatum* is related to the age of the workers and the ovarian cycle described by Fénéron and Billen (1996). Our data showed that vitellogenin appears in the haemolymph of workers around the fifth day after emergence, being secreted in quantities not detectable by SDS-PAGE. The age at which the ovaries of workers of *E. tuberculatum* begin to be activated is variable, since at the end of the first week after adult emergence workers can be found that either have ovarioles without follicles and only undifferentiated cells or ovarioles with oocytes in the early accumulation of vitellogenin (Fénéron and Billen, 1996). Vitellogenin production remains low until the second week after emergence. At the 20th day it is present in large amounts in the haemolymph, at which time the workers have developing oocytes (Fénéron and Billen, 1996). The vitellogenin is still present in the haemolymph of workers aged 30 and 60 days, which is the period of maximum development of oocytes and subsequent oviposition (Fénéron and Billen, 1996). Oocytes can still be found in workers 90 days old, but the ovaries are already in the regression stage (Fénéron and Billen, 1996). The vitellogenin is not present in the haemolymph of workers with more than 100 days of age, during which time workers have degenerated ovaries (Fénéron and Billen, 1996).

The secretion of vitellogenin is another factor to be considered in the maintenance of age polyethism in *E. tuberculatum*. In most species of ants, the young workers start their tasks inside the colony and when older perform other tasks outside the colony (Hölldobler and Wilson, 1990). In *E. tuberculatum*, the workers have this same pattern of activity, with a gradual progression of inside colony tasks to outside colony tasks as they age (Champalbert and Lachaud, 1990; Fénéron et al., 1996). In the first week of adult life, the workers are involved in reconnaissance activities directed towards nestmates and brood and are not performing any specific function inside the colony. From the second week, the workers direct their care to the eggs, larvae and pupae, which may be considered nursing activities. At around 30 days, the workers' main activity remains the care of brood, but they begin non-specific activities like exploring the colony. The workers around 90 days of age perform the tasks of cleaning and maintaining the colony, guarding and foraging (Champalbert and Lachaud, 1990; Fénéron et al., 1996). Our results show that vitellogenin is not produced during the stabilization of the social interactions of newly emerged workers. Instead its synthesis begins when workers start brood care activities, is maintained while workers act as nurses and ends when workers begin to forage.

The interruption of vitellogenin synthesis in workers of *E. tuberculatum* at around 100 days of age may be a trigger for the beginning of outside colony activities. Worker ants that perform activities outside of the colony are more at risk of death by external factors than those who remain within the colony. Therefore, they show greater rates of aging and a shorter lifespan (Chapuisat and Keller, 2002; Keller and Genoud, 1999). In the weaver ant *Oecophylla smaragdina* (Formicidae: Formicinae) the *minor* workers that remain within the colony are subject to lower rates of extrinsic mortality and showed greater longevity than the caste of *major* workers, responsible for activities outside the colony (Chapuisat and Keller, 2002). In *A. mellifera*, the inhibition of vitellogenin production causes the workers to begin foraging flights earlier, an activity characteristic of older workers (Marco Antônio et al., 2008). The reduced longevity of individuals at higher risk of extrinsic mortality saves energy and resources for the colony since it is more advantageous to invest resources into somatic maintenance of organisms that have a greater chance of survival (Chapuisat and Keller, 2002).

Based on this theory, the onset of foraging activities of worker ants and bees is linked to a decline of their physiological functions and to an increased chance of extrinsic mortality. The interruption of the production of vitellogenin negatively affects an insect's body since it may compromise its immunity and resistance to oxidative stress (Amdam et al., 2004; Corona et al., 2007), both of which promote aging (Muller et al., 2007).

5. Conclusion

In conclusion, the production of vitellogenin by workers of *E. tuberculatum* is age dependent and related to the performance of various tasks by this caste.

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