Insect Biochemistry and Molecular Biology 115 (2019) 103241

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb

# Telomerase activity is upregulated in the fat bodies of pre-diapause bumblebee queens (*Bombus terrestris*)

Justina Koubová[a,b], Tomáš Jehlík[a,b], Dalibor Kodrík[a,b], Michala Sábová[a], Peter Šima[c], Hana Sehadová[a,b], Radka Závodská[a,d], Radmila Čapková Frydrychová[a,b,*]

[a] Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Branišovská 31, 370 05, České Budějovice, Czech Republic
[b] Faculty of Science, University of South Bohemia, Branišovská 31, 370 05, České Budějovice, Czech Republic
[c] Koppert s.r.o., Komárňanská cesta 13, 940 01, Nové Zámky, Slovakia
[d] Faculty of Pedagogy, University of South Bohemia, Branišovská 31, 370 05, České Budějovice, Czech Republic

ARTICLE INFO

*Keywords:*
Telomerase
Telomeres
Ageing
Social insect
Bombus

ABSTRACT

The attrition of telomeres, the ends of eukaryote chromosomes, and activity of telomerase, the enzyme that restores telomere length, play a role in the ageing process and act as indicators of biological age. A notable feature of advanced eusocial insects is the longevity of reproductive individuals (queens and kings) compared to those from non-reproductive castes (workers and soldiers) within a given species, with a proposed link towards upregulation of telomerase activity in the somatic tissues of reproductive individuals. Given this, eusocial insects provide excellent model systems for research into ageing. We tested telomerase activity and measured telomere length in *Bombus terrestris*, which is a primitively eusocial insect species with several distinct features compared to advanced social insects. In somatic tissues, telomerase activity was upregulated only in the fat bodies of pre-diapause queens, and this upregulation was linked to heightened DNA synthesis. Telomere length was shorter in old queens compared to that in younger queens or workers. We speculate that (1) the upregulation of telomerase activity, together with DNA synthesis, is the essential step for intensifying metabolic activity in the fat body to build up a sufficient energy reserve prior to diapause, and that (2) the lifespan differences between *B. terrestris* workers and queens are related to the long diapause period of the queen. A possible relationship between telomere length regulation and TOR, FOXO, and InR as cell signaling components, was tested.

## 1. Introduction

Eusociality is an evolutionarily advanced level of colony organization, where some individuals reduce their own reproductive potential to raise the offspring of others (Nowak, 2010; Szathmáry and Smith, 1995; West et al., 2015). The best-known animal group with eusocial species is the order Hymenoptera. The advanced eusocial species within Hymenoptera, such as bees, ants, and wasps consist of large perennial colonies formed of semi-autonomous individuals, where females are morphologically, physiologically, and behaviorally differentiated as either reproductive queens or non-reproductive workers (Engels, 1990).

A notable feature of advanced eusocial insects is the difference in lifespan between reproductive and non-reproductive castes within a given species. Although there are no substantial genetic differences between the castes, the lifespan of reproductive individuals is up to 100-fold longer than that of non-reproductive individuals, with the recorded 4–8 year lifespan of bee queens and the 18–30 year lifespan of ant queens (Carey, 2001). Given this, eusocial insects provide excellent model systems for research into ageing.

Ageing is characterized by a progressive and time-related accumulation of diverse deleterious changes in cells and tissues with advancing age that lead to impaired function of the organism and its increased susceptibility to disease and death. Numerous theories have been proposed as universal clues to the ageing process, but none of the theories appear to be fully satisfactory (Davidovic et al., 2010; Jin, 2010).

Prolonged lifespan and resistance to age-associated diseases is associated with telomeres and telomere maintenance mechanisms. Telomeres, the special nucleoprotein structures at chromosome ends, are essential to solve the so called chromosome end shortening problem (Chan and Blackburn, 2002; Denchi, 2009). Cellular processes, such as incomplete DNA replication or oxidative damage, lead to a gradual loss of chromosome termini, ultimately resulting in senescence or apoptosis. The most common mechanism of compensation for telomere shortening is the activity of telomerase, an enzyme that adds short repetitive DNA

* Corresponding author. Institute of Entomology, Biology Centre AS CR, Branišovská 31, České Budějovice, 37005, Czech Republic.
*E-mail address:* Radmila.Frydrychova@hotmail.com (R.Č. Frydrychová).

https://doi.org/10.1016/j.ibmb.2019.103241
Received 18 July 2019; Received in revised form 6 September 2019; Accepted 9 September 2019
Available online 16 September 2019

sequences to the chromosome ends (Mason et al., 2016). Telomerase activity correlates with cell proliferation, and the highest activity of telomerase has been found in rapidly dividing embryonic cells, germ cells, and stem cells (Cong et al., 2002; Wright et al., 1996). Dysfunction of telomerase is linked to ageing processes and cancer (Aubert and Lansdorp, 2008), and the length of telomeres might be used as a predictor of lifespan (Heidinger et al., 2011). Decline of telomerase activity is a marker of ageing and advancing organismal development, seen not only in vertebrates, but also in insects (Korandová et al., 2014). Nevertheless, telomerase activity in the somatic tissues of honeybees and termites is strongly upregulated compared to that of workers [16, unpublished data], suggesting that upregulation of telomerase activity may be linked to the longevity of reproductive individuals of eusocial insects.

*Bombus terrestris* (Hymenoptera: Bombini) is a hymenopteran species with primitive social organization and, compared to advanced social insects such as *Apis mellifera*, have several distinct features. For instance, colonies of *B. terrestris* are annual and have smaller population sizes than those of more advanced social insects. After a relatively long diapause, their queens undergo both solitary and social phases. In general, bumble bee queens are only slightly morphologically different from workers (Bloch, 1999; Bloch and Hefetz, 1999; Goulson, 2010).

The main goal of this study was to map telomerase activity in *B. terrestris* to see if this species, despite its primitive social organization, has the same pattern in telomerase activity as observed in *A. mellifera*. Levels of telomerase activity in the gonads and somatic tissues of workers, young (pre-diapause), and older (reproductive) queens, were evaluated. As cell signaling is an important player in longevity regulation, we also investigated this phenomenon in a relationship with telomere length regulation.

## 2. Materials and methods

### 2.1. Bumblebee rearing and sample collection

For experimental purposes, fifty queenright colonies of *Bombus terrestris* (Koppert Biological Systems, Slovakia) were used. Colonies were kept in plastic hives under environmentally controlled conditions at 28 ± 2 °C and relative humidity of 50–60%. A sugar solution (mixture of glucose, fructose, and sucrose, 48 °Brix) and fresh, frozen honeybee corbicular pollen was administered *ad libitum* during the rearing process.

Virgin queens and males were collected from the colonies 24 h post-eclosion and kept in plastic nest boxes (7.5 × 17.5 × 16 cm) with an *ad libitum* supply of sugar solution and bee pollen until tested. Mated queens were prepared by copulation of 9–11-day-old queens with unrelated males of the same age. To induce diapause, the mated queens were gradually cooled along a temperature gradient, until transferred to a cooler (3.5 ± 1 °C, > 90% relative humidity, 24 h darkness). For the experiments with diapause queens, copulated individuals kept in diapause for 8 days, and 12 and 24 weeks, were used. To test copulated reproducing queens, we used post-diapause 7-month- and 45–48-week-old (further mentioned as 1-year-old) queens. The 7-month-old queens were collected from developing colonies during the solitary phase. The 1-year-old queens were collected from colonies in terminal phase, after the switch and competition points. Workers were collected from colonies either 24 h post-eclosion or randomly at 1–3-week-old. Ovipositing workers were collected during observed oviposition from 11-week-old queenright colonies after the competition point. For telomerase activity evaluation, we collected post-eclosion males and kept them in plastic boxes for 14 days. During the ageing time, sugar solution and pollen was administered *ad libitum*.

### 2.2. Evaluation of telomerase activity

For evaluation of telomerase activity we used 24-h-old virgin queens; 10-day-old queens, which were 24 h post copulation, and 10-day-old virgin queens; 4-week-old queens, which were 3 weeks post copulation; diapausing queens; 7-month and 1-year-old reproducing queens; 2-h and 1–3-week-old workers; ovipositing workers; 24-h and 2-week-old males, and embryos. Up to 50 µl of dissected tissue was homogenized in 200 µl of extraction buffer (10 mM Tris/HCl, pH 7.6; 1 mM $MgCl_2$; 1 mM EGTA; 0.1 mM benzamidine (PMSF); 5 mM 2-mercaptoethanol; 0.5% (w/v) CHAPS; 10% (v/v) glycerol, and 40 U/ml RNase inhibitor (Promega). The homogenized samples were incubated on ice for 30 min, then centrifuged (12,000 *g*; 20 min; 4 °C), and collected supernatants were stored at −80 °C until further use. Total protein concentration was determined using a BCA protein assay reagent kit (Pierce).

Telomerase activity was determined using a Telomerase repeat amplification protocol (TRAP) assay as described earlier (Korandová et al., 2014). Briefly, the reaction mixture was first incubated at 30 °C for 60 min to allow telomerase to extend the TS primer (5′- AATCCGT CGAGCAGAGTT - 3′) by adding a TTAGG repeat. The extension products were amplified by PCR with TS primer and Bm-CXa primer ( 5′-GTGTAACCTAACCTAACC-3′). Each reaction was performed in 25 µl total volume and contained 5 ng of protein, 5 pmol of both TS and Bm-CXa primer, and 12.5 µl Xceed qPCR SG 2 × Mix (Institute of Applied Biotechnologies). Samples were incubated at 30 °C for 60 min, then PCR was performed with 30 cycles at 94 °C for 30 s and 60 °C for 30 s. Telomerase activity quantitation was accomplished using a Light Cycler CFX96 BioRad Real-time PCR system (BioRad). For each tested extract, we prepared negative controls for telomerase-specific activity by incubation of protein extracts with 0.5 µl of 1 µg/µl RNase A (Sigma-Aldrich) for 20 min at 37 °C prior to TS primer elongation.

### 2.3. Cloning and sequencing of TRAP products

The TRAP products were purified with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel) and cloned into pGEM®-T easy vector (Promega). After isolation of plasmid DNA (Nucleospin Plasmid Quickpure Kit, Macherey-Nagel), the inserts were sequenced using ABI PRISM 3.1 (Applied Biosystems) using T7 and SP6 primers.

### 2.4. Evaluation of telomere length: DNA isolation and Southern hybridization

Telomere length was tested in 24-h- and 1-year-old queens, and in ovipositing and 1–3-week-old workers. Genomic DNAs from the tested samples were isolated by the standard procedure of phenol:-chloroform:isoamyl alcohol extraction. DNA samples (1 µg) were digested with the restriction enzymes RsaI and Hinf I (New England Biolabs) to produce terminal restriction fragments. Following DNA digestion, the DNA fragments were separated on 1% agarose gel using a pulsed-field gel electrophoresis systems (Bio-Rad), examined by ethidium bromide staining to check for the absence of unspecific degradation and complete digestion, and transferred to a positively charged nylon membrane (Hybond-$N^+$; GE Healthcare) by Southern blotting. The $(TTAGG)_n$ hybridization probes were prepared by the non-template polymerase chain reaction (PCR) method using $(5'\text{-}TTAGG\text{-}3')_5$ and $(5'\text{-}CCTAA\text{-}3')_5$ primers. PCR was performed in 100 µl total volume and contained 0.5 µM of each primer, 0.8 mM dNTP, and 5U of Taq polymerase (Takara). Thermal cycling conditions consisted of 90 s at 95 °C, followed by 30 cycles of 45 s at 94 °C, 30 s at 52 °C and 60 s at 72 °C. A final, 10 min elongation step was performed at 72 °C. PCR products were purified using ethanol precipitation and labeled by Nick-translation with $^{32}$P-dCTP using Nick translation system (ThermoFisher Scientific). Experiments were conducted with three different DNA samples of each tested tissue or stage.

To confirm telomeric positions of (TTAGG)n sequences, the genomic DNA was digested with BAL 31 nuclease, which progressively degrades linear DNA from both the 5′ and 3′ ends. Genomic DNA (15 µg) was

treated with 0.03 U BAL 31 nuclease (New England Biolabs) in a total volume of 180 µl at 30 °C. The BAL 31 digestion was monitored by taking a 60 µl aliquot before addition of BAL 31, and again after 30 and 60 min of treatment. Digestion was stopped by incubation at 65 °C for 10 min in the presence of EGTA. Samples were purified using phenol:-chloroform:isoamyl alcohol extraction, then 1 µg of DNA from each tested sample was digested with the restriction enzymes RsaI and Hinf I (New England Biolabs) and subjected to Southern hybridization.

### 2.5. Gene expression analysis

Gene expression analysis was done in 24-h-old virgin queens, 10-day-old mated queens, reproducing 1-year-old queens, ovipositing workers, and 1–3-week-old workers. Total RNA from the samples were prepared using Hybrid-R (GeneAll), and cDNA synthesis was performed using 1 µg of total RNA primed with oligo(dT) and 2 × HyperScript Master mix (GeneAll). To evaluate relative transcript levels of tested genes, we performed qRT-PCR as described previously (Jedlicka et al., 2016; Korandová et al., 2018). We used a Light Cycler CFX96 Bio-Rad Real-time PCR system and Xceed (IAB). To obtain relative expression values for each target gene, generated Ct values were calibrated against the geometrical mean of phospholipase A2 and elongation factor EF-1. The reference genes were selected based on a previous study (Jedlicka et al., 2016), and the Comparative CT Method, with correction for amplification efficiency, was used to calculate levels of the targets. Sequences of primers are shown in Table S1.

### 2.6. Detection of DNA replication in the fat bodies

The Click-iT EdU Alexa Fluor 488 Imaging Kit (Invitrogen by Thermo Fisher Scientific) was used for detection of S-phase nuclei in the fat bodies. The analysis was performed in 10-day-old and 18-day-old virgin queens, in 10-day-old and 18-day-old mated pre-diapause queens, in 1-year-old reproductive queens, in workers, and in ovipositing workers. The fat bodies were dissected in M3 Insect Medium (Sigma-Aldrich), then cultivated for 24 h in the same media supplemented with fetal bovine serum (12%), insulin (0. 1%), penicillin/streptomycin solution (1%), and 0.4 mM EdU (5-ethynyl-2′deoxyuridine). Samples were fixed in 3.7% formaldehyde in phosphate-buffered saline (PBS) for 2 h, washed in PBS three times (for 15 min each), and finally washed in PBS, 0.5% v/v Triton X-100 for 15 min. Samples were incubated with 3% w/v bovine serum albumin in PBS for 30 min and then in Click-iT reaction cocktail for 12 h at 4 °C. After washing in PBS (three times for 15 min), the samples were incubated in a solution of 4′-6-diamidino-2-phenylindole (DAPI, 1 µg DAPI in 1 ml distilled $H_2O$). Samples were then dehydrated in ethanol (50, 70, 90, and 100%), mounted in methyl-salicylate (Sigma-Aldrich), and inspected under a confocal microscope (Olympus FV-1000).

### 2.7. Spectrophotometric determination of nutrients

Nutrient levels were determined in virgin and mated pre-diapause queens, which were both 10 days and 18 days old, in 1-year-old reproductive queens, in workers, and ovipositing workers. The fat body of each experimental bumblebee was dissected in Ringer's saline using a dissecting microscope, weighted, and stored at −20 °C until used.

For protein determination, the fat bodies were homogenized in 0.2 M Tris-HCl buffer, pH 7.8, and after centrifugation the supernatants were used for protein quantitation using the Bicinchoninic Acid Protein Assay Kit (Sigma Aldrich) (Stoscheck, 1990). The optical densities of tested samples were measured at 562 nm and, using the bovine serum albumin standard curve, converted to µg of proteins.

For lipid determination, the fat bodies were homogenized in a chloroform:methanol (2:1) mixture (Folch et al., 1957; Kostal and Simek, 1998). After evaporation in a SpeedVac centrifuge (Jouan RC 10.22), the residues were used for total lipid determination using the sulfo-phospho-vanillin method (Goldsworthy et al., 2002; Zöllner and Kirsch, 1962). The optical densities at 546 nm were measured spectrophotometrically and were converted to µg of lipids using a calibration curve with specified doses of oleic acid.

For glycogen determination, the fat bodies were homogenized in 70% ethanol, and glycogen levels were determined as described previously (Socha et al., 2004).

### 2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) by one-way ANOVA followed by Tukey's multiple comparison test. The number of replicates is specified in the figure legends, and the bars in graphs represent the mean ± SD.

## 3. Results

### 3.1. Telomerase activity

The first series of experiments were focused on telomerase activity, and its relative levels were analyzed in the gonads and somatic tissues of adult queens, males, workers, and in embryos. Ovaries generally had the highest level of telomerase activity, but this was influenced by the postmating time interval, diapause, age, and ovarian regions (Fig. 1). The highest telomerase activity in ovaries was observed in germarium of reproducing queens (7-month- and 1-year-old queens) and ovipositing workers, which were a nearly 500-fold higher ($P < 0.01$) in telomerase activity, compared to that in virgin queens, or 100-fold higher compared to that in embryos. A comparison of telomerase activities between embryos and testes revealed statistically comparable levels (Figure S1). We found no telomerase activity in hemolymph (not shown) and low levels without significant differences between the heads and leg muscles in experimental bumblebees. Telomerase activity in the heads and leg muscles was nearly 4-fold lower to that in embryos (Fig. 2A and B). In contrast, a high level of telomerase activity was detected in the fat bodies of pre-diapause queens (Fig. 2C), where 1-day-old and 10-day-old virgin representatives showed a nearly 10-fold higher activity ($P < 0.01$) compared to that in embryos. Interestingly, only a 3-fold higher telomerase activity was found in 10-day-old mated queens (24 h after copulation), perhaps indicating that telomerase activity is influenced by the reproduction process.

Specificity of the TRAP reaction for $(TTAGG)_n$ repeats was confirmed by cloning and sequencing of TRAP products.

### 3.2. DNA synthesis in the fat body is strongly upregulated during early age of pre-diapause queens

Our next goal was to determine whether the upregulation of telomerase activity observed in the fat bodies of pre-diapause queens is associated with increment of DNA replication. Presence of DNA replication, and its intensity, was visualized using EdU (5-ethynyl-2′deoxyuridine), and the assay was performed in workers, pre-diapause, and 1-year old reproductive queens. To evaluate whether DNA synthesis varies depending on mating status, virgin and mated pre-diapause queens of the same age (10-day-old and 18-day-old) were assessed.

The fat body tissues consisted of two cell types, adipocytes and oenocytes (Fig. 3). Oenocytes surrounded adipocytes, their nuclei were roundish and compact (Fig. 3A). However, nuclei of adipocytes were of irregular shape, as they were compressed by numerous vacuoles, and showed dispersed chromatin (Fig. 3B). The size of adipocytes varied in a caste- and age-dependent manner; larger adipocytes were found in pre-diapause queens than in post-diapause queens or workers (Fig. 4, S2). There were considerable differences in the presence and localization of DNA replication between tested representatives in both adipocytes and oenocytes (Fig. 4). Strong EdU staining was observed as a

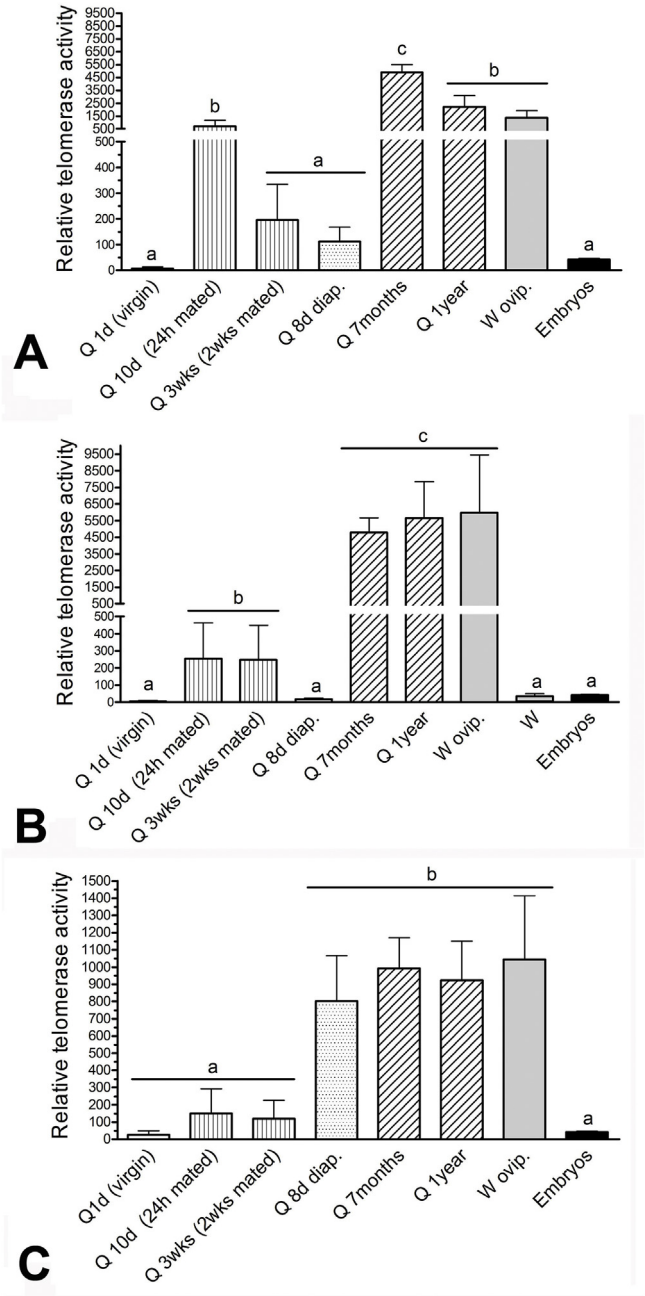



**Fig. 1.** Levels of telomerase activity in ovaries. Relative telomerase activity was quantified using TRAP assay in workers, ovipositing workers, and in queens of different ages and status in terminal filament with distal part of germarium **(A)**, middle and proximal parts of germarium **(B)** and vitellarium **(C)**. Embryo samples were included in each experiment as references, and each embryo sample was prepared from five embryos. Q1d (virgin) – 1-day-old virgin queens; Q 10d (24 h mated) – 10-day-old queens that were 24 h after mating; Q 3wks (2wks mated) – 3-week-old queens that were two weeks after mating; Q 8d diap. – queens that were in diapause for eight days; Q 7months – 7-month-old queens; Q 1year – 1-year-old queens; W ovip. – ovipositing workers; W – workers. Statistical significance was determined using One-way ANOVA and Tukey's post-hoc tests ($p < 5\%$, indicated by different letters above columns); $n = 5$. The bars in graphs represent the mean ± SD.

general feature of both cell types in 10-day-old pre-diapause queens and no difference in intensity or localization of Edu signal was found between mating statuses (data for virgin queens are presented in Fig. 4A and B; data for mated queens are not shown). Adipocytes showed signals formed by numerous points scattered along the chromatin, whereas oenocytes revealed dense EdU signals covering the whole area of the nuclei. Nevertheless, when we inspected the character of EdU signals in oenocytes using less intense confocal microscopy laser, the EdU signal in oenocyte nuclei was formed by numerous strong dots (the detailed section in Fig. 4B). In contrast, in 18-day-old pre-diapause queens, EdU staining was absent or in some cases, very faint (Fig. 4D, where the fat body of a virgin queen is presented). In 1-year-old queens, EdU staining was generally hardly observed and only as minute dots in oenocytes. However, we occasionally found spots with intense staining in both cell types (Fig. 3F). No EdU signal was detected in workers or ovipositing workers (data not shown).

### 3.3. Telomere length in the fat body is shortened in 1-year-old queens

In the next step, we evaluated whether DNA replication in the fat bodies of 1-year-old queens, although observed at some level, but without upregulated telomerase activity, could be reflected by shortening of telomere length in aged queens. To measure telomere length, we used Southern hybridization of terminal restriction fragments, and evaluated the position and intensity of hybridization signals. The hybridization signals were revealed as faint smears with numerous discrete bands ranging from approximately 3 kb to more than 48 kb (Fig. 5), and the telomeric origin of hybridization bands was confirmed by Ball digestion (Figure S3). There were no obvious differences in overall size and intensity in hybridization bands between virgin queens and workers, but hybridization bands in 1-year old queens were weaker, blurrier, and showed lower molecular weight (Fig. 5).

### 3.4. The fat body mass and energy reserves are elevated in pre-diapause queens

To answer the question whether upregulation of telomerase activity and DNA synthesis in the fat bodies of young pre-diapause queens is followed by increased metabolic activity, we evaluated the total mass, lipid, glycogen, and protein levels in the fat bodies of queens and workers. To detect possible effects of mating status on the tested characteristics, we compared virgin and mated queens of the same age (10-day-old and 3-week-old). The fat body mass was the largest in pre-diapause queens. Compared to workers, 5-fold ($P < 0.001$) differences were observed in 10-day-old and 3-week-old queens, and 3-fold ($P < 0.001$) differences were found in 1-day-old virgin queens. In contrast, in 1-year-old reproducing queens the fat body mass was lower and comparable to that of workers (Fig. 6A). The greatest lipid content, both lipid amounts and concentrations (per unit of fat body mass), was found in pre-diapause queens (Fig. 6B and C), and the values in pre-diapause queens were age-dependent. While in 1-day-old queens, both the lipid characteristics were statistically comparable to those in workers or post-diapause queens, 3-week-old queens had a nearly 10-fold higher level of lipids ($P < 0.001$) than that of workers. A similar trend was observed in total glycogen amounts as they showed comparable levels between 1-day-old queens and post-diapause queens or workers, and higher amounts in older pre-diapause queens (Fig. 6D). The amount of glycogen in 3-week-old queens was 5-fold higher ($P < 0.001$) than in workers. However, this pattern was lost when glycogen concentrations in the fat bodies were evaluated. Here, a significantly lower level was found in 1-day-old queens compared to those in workers or older queens. Analysis of protein levels showed no clear pattern(Figure S4). Interestingly, the Student's t-test showed that lipid and glycogen concentrations were significantly lower in mated queens than in 3-week-old virgin queens (1.5-fold, $P < 0.04$ for lipids, and 1.4-fold, $P < 0.03$ for glycogen) (Fig. 6C, E).

### 3.5. Alteration in the cell signaling in queens due to changes in telomere length regulation and metabolic activity in their fat bodies

Finally, we evaluated whether changes observed between the fat

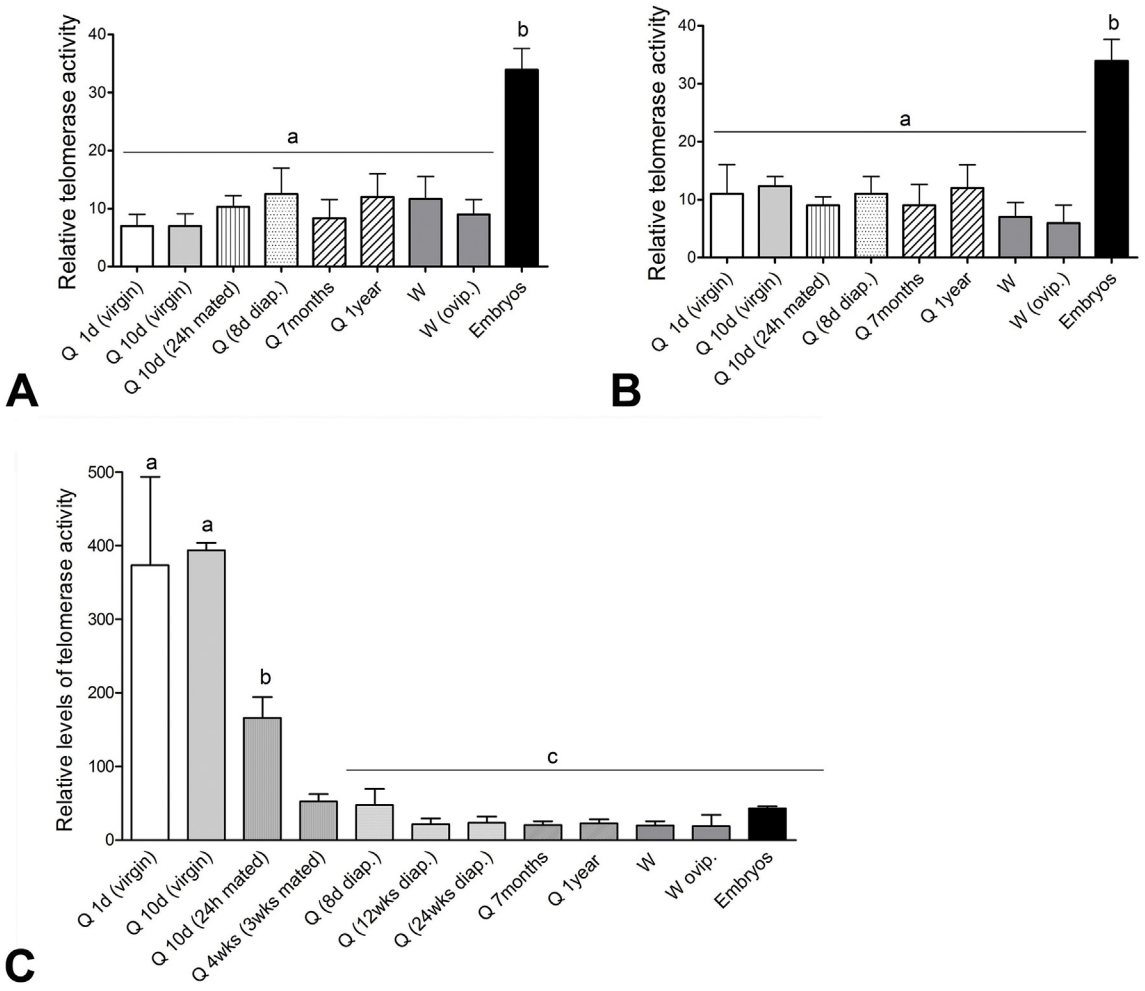



**Fig. 2.** Levels of telomerase activity in the somatic tissues. Relative telomerase activity was quantified using TRAP assay in queens of different ages and status, in workers and ovipositing workers in heads **(A)**, leg muscles **(B)** and the fat bodies **(C)**. Embryo samples were included in each experiment as references **(A–C)**, and each embryo sample was prepared from five embryos. Q1d (virgin) – 1-day-old virgin queens; Q 10d (virgin) – 10-day-old virgin queens; Q 10d (24 h mated) – 10-day-old queens that were 24 h after mating; Q 4wks (3wks mated) – 4-week-old queens that were three weeks after mating; Q (8d diap.) – queens that were in diapause for eight days; Q (12wks diap.) – queens that were in diapause for 12 weeks; Q (24wks diap.) – queens that were in diapause for 24 weeks; Q 7months – 7-month-old queens; Q 1year – 1-year-old queens; W ovip. – ovipositing workers; W – workers. Statistical significance was determined using One-way ANOVA and Tukey's post-hoc tests ($p < 5\%$, indicated by different letters above columns); $n = 5$. The bars in graphs represent the mean ± SD.

bodies of young and old queens might be associated with alterations in cell signaling. First, we tested transcript levels of vitellogenin (*Vg*). Vg is the protein synthesized at high levels in the fat body during vitellogenesis of reproducing females, but it has also been proposed as a determinant of lifespan extension in honeybee queens (Corona et al., 2007). Consistent with the role of Vg in reproduction, we found its highest levels in ovipositing workers (up to 300-fold higher than that in workers, $P < 0.001$) and 1-year-old queens (100-fold higher than that

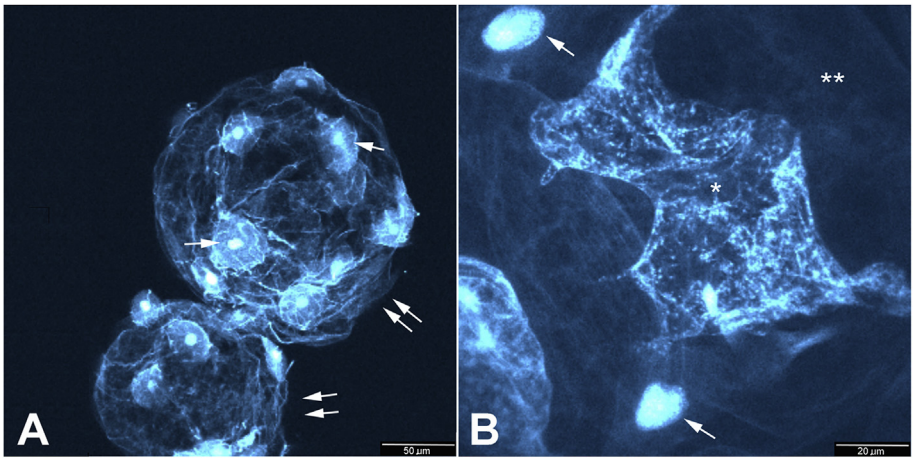



**Fig. 3.** The fat body of *B. terrestris* is composed of adipocytes and oenocytes. **A.** Oenocytes (indicated by arrows) surround adipocytes (indicated by double-arrows). **B.** Nuclei of oenocytes are roundish and compact (indicated by arrows), adipocytes possess nuclei of irregular shape (indicated by single-stars). Area of adipocyte cytoplasm is indicated by double-stars. The presented nuclei represent nuclei of 10-day-old queens.

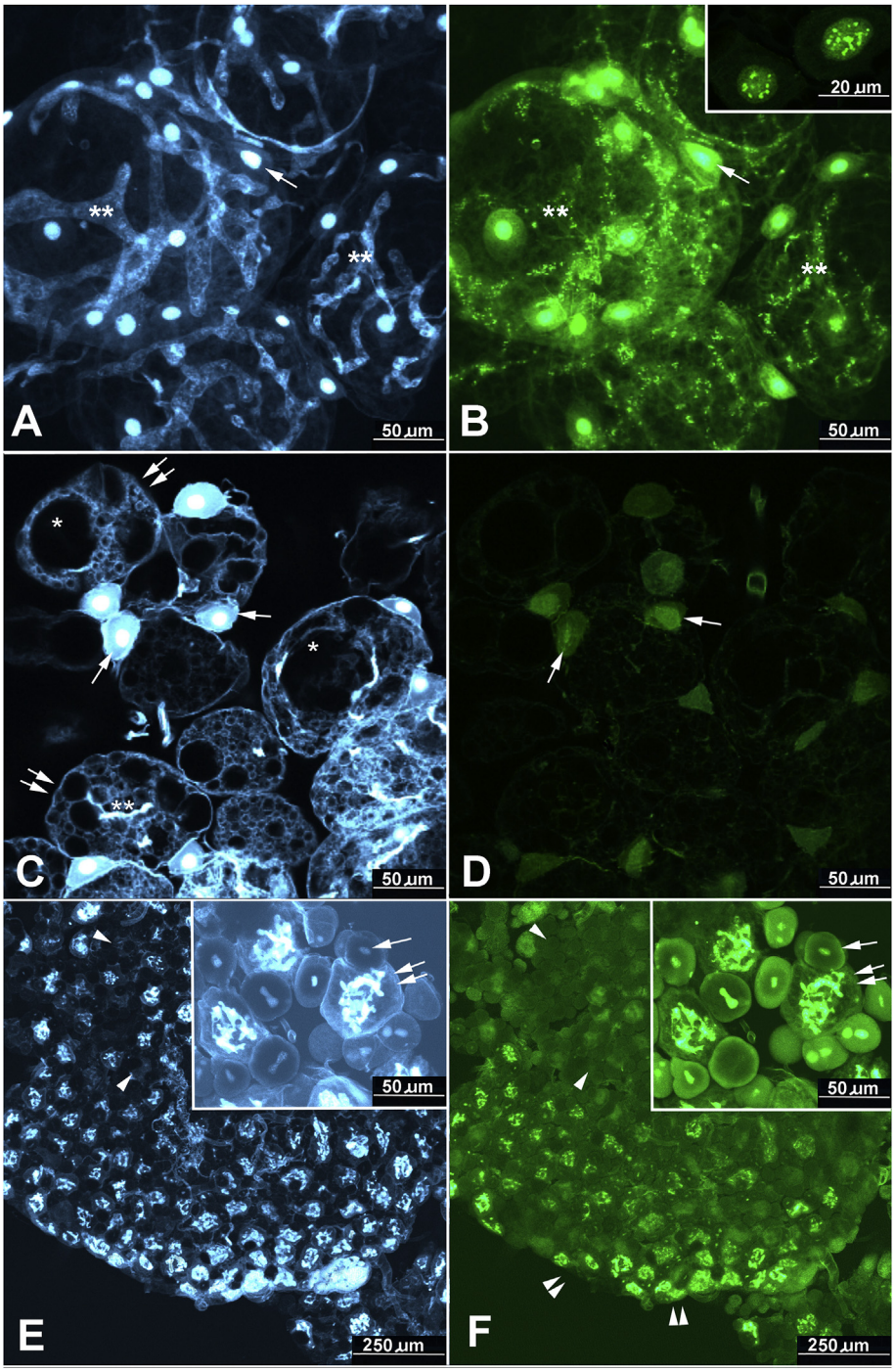



**Fig. 4.** S-phase nuclei in the fat bodies of queens and workers. S-phase nuclei were visualized using thymidine analogue EdU incorporated into newly synthesized DNA and fluorescently labeled with Alexa 488 (green signals). DNA was labeled with DAPI (blue signals). The assay was performed in 10-day-old virgin queens **(A, B)**, in 18-day-old virgin queens **(C, D)** and 1-year-old queens **(E, F)**. Arrows, oenocytes; double-arrows, adipocytes; stars, lipid droplets; double-stars, adipocyte nuclei; arrowheads, area of the fat body without S-phase nuclei; double-arrowheads, area of the fat body with S-phase nuclei. Sections in B, E, F show character of DAPI or EdU signals in more detail.

in workers, $P < 0.001$), and no significant differences were seen in *Vg* transcript levels between workers and young queens (Fig. 7A).

Next, we tested transcript levels of FOXO, TOR, and InR, the factors involved in cell cycle growth regulation, and lifespan extension (Blagosklonny, 2010; Hay, 2011). The fat bodies of 1-year-old queens showed the highest level of TOR compared to that of young queens or workers (nearly 25-fold higher, $P < 0.05$) and the lowest level of FOXO (without statistical significance) (Fig. 7B and C). When compared young and old queens, FOXO and TOR levels reveal a positive and negative correlation to telomerase activity, respectively. We then tested whether the same trend between telomerase activity and the FOXO/TOR transcripts might be observed in ovaries, and found that 1-year-old queens manifested the highest levels of FOXO (around 10-fold higher than in virgin queens, $P < 0.05$) and lowest levels of TOR (around 20-fold lower than in virgin queens, $P < 0.01$) (Fig. 8). When we tested transcript levels of InR in both the fat body and ovaries, the data were inconclusive and not included in further consideration (data not shown).

## 4. Discussion

To explain biological ageing, a number of theories have been proposed, explaining ageing as a gradual process of damage to cellular functions or as a genetically programmed and necessary biological function (Sergiev et al., 2015). According to the programmed theory, ageing is controlled by a complex regulation system involving, for instance, changes in gene expression and cell signaling, or a decline in telomerase activity and immune response (Jin, 2010; Sergiev et al.,

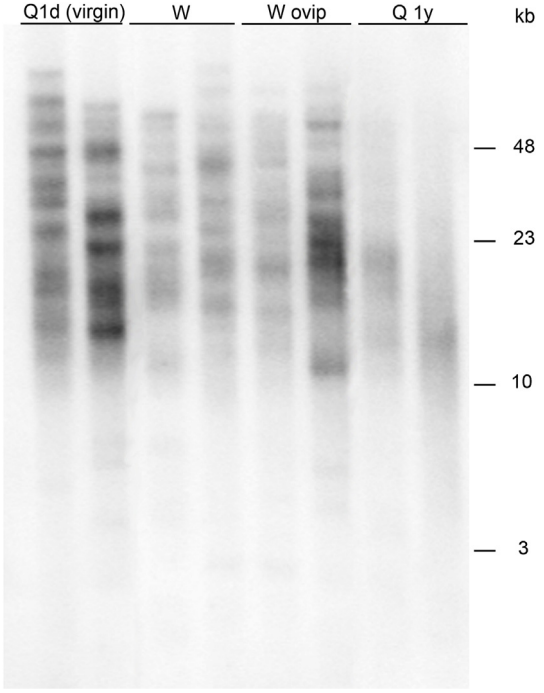



**Fig. 5.** Telomere length in the fat body. The (TTAGG)n sequences were visualized using Southern hybridization with a probe labeled with $^{32}$P-dCTP. W, workers; W ovip, ovipositing workers; Q1d (virgin), 1-day-old virgin queens; Q 1y, 1-year-old queens.

2015). Based on the disposable soma theory of ageing (Kirkwood and Holliday, 1979), the organism's lifetime is inversely correlated with reproduction rate/potential of the individual in relation to differential allocation of resources between somatic maintenance and the reproductive process. Although this phenomenon is generally valid, it is not universal, as shown in eusocial insects or mammals, such as naked mole rats (Bens et al., 2018; Jemielity et al., 2005; Lucas and Keller, 2014).

The differences in life expectancy in relation to the social/reproductive status of the individual, and contradiction of the disposable soma theory, make eusocial insects an excellent model system to study the regulation of ageing. The exact mechanisms determining lifespan differences in social organisms are unknown, but some changes have been found at the expression level of ageing-related genes associated with lipid metabolism, oxidative phosphorylation (Bens et al., 2018), antioxidant protection (Corona et al., 2005), and mitochondrial maintenance (Aamodt, 2009), directed by reprogramming of the genome by epigenetic modifications (Chittka and Chittka, 2010; He et al., 2017; Herb et al., 2012; Kucharski et al., 2013). Telomere attrition, together with low telomerase activity may contribute to organismal ageing by limiting stem cell division potential, leading to a progressive loss of tissue and organ regeneration capacity, which in turn limits organismal longevity (Flores and Blasco, 2010; Milewski, 2010; Rodier et al., 2007).

In our previous studies, we tested telomerase activity in several species of eusocial insects, such as honeybees and several termite species, to determine whether caste-related lifespan differences correlate with levels of telomerase activity, and indeed, in all of the species, we consistently found elevated telomerase activity in the somatic tissues of reproductive individuals ((Korandova and Frydrychova, 2016); data on termite species in unpublished studies).

In *B. terrestris* the caste-related lifespan differences are less pronounced than in advanced eusocial species (Lin and Michener, 1972). Although *B. terrestris* workers live for 2–3 months and queens can live up to 1 year, it needs to be considered that queens spend most of their life (6–9 months) in diapause (Alford, 1969a; Lopez-Vaamonde et al., 2009; Smeets and Duchateau, 2003). A key requirement to survive diapause is a sufficient energy reserve, which is built up and stored in the fat body during a limited time prior to diapause (Holm, 1972). The insect fat body is a major energy storage organ with a high metabolic activity, and in *B. terrestris* queens it changes significantly in structure and function over the course of their lifetime (Votavová et al., 2015). After emergence from the pupa, the queen's fat body develops (Alford, 1969b), and considerable energy reserves are synthesized and stored in adipocytes, most of which are utilized during diapause (Alford, 1969b). In young, pre-diapause queens, we found up-regulated telomerase activity with DNA replication, while the highest levels of fat body mass and lipid reserves in queens were found at the end of the pre-diapause period. In contrast, the fat body mass, lipid reserves, and adipocyte size were low in post-diapause queens and in workers, which is consistent with previous observation (Votavová et al., 2015). Regarding the polyploid character of the fat body cells, DNA synthesis signals observed in our experiments come from DNA endoreduplication cycles rather than from cell proliferation. DNA endoreduplication is a cellular mechanism employed to maximize energy production and storage away from costly processes such as mitosis or cytokinesis (Lee et al., 2009). In view of the crucial role of adipocytes and oenocytes in lipid processing (Martins and Ramalho-Ortigão, 2012), we can speculate that upregulation of telomerase activity and increased DNA replication are prerequisites for the high metabolic activity needed in these tissues for upcoming diapause. Interestingly, telomerase activity in bumblebee queens, like lipid and glycogen levels, seems to decrease after mating; however, to get complete clarification of this finding, more detailed studies should be conducted.

Among tested somatic tissues of *B. terrestris*, the upregulation of telomerase activity found only in the fat body and only in young pre-diapause queens contrasts substantially with that of species of advanced eusocial insects, as these species show elevated levels of telomerase activity in various somatic tissues, independently of age (Korandova and Frydrychova, 2016). In contrast to our observation of telomere shortage in 1-year-old queens, previous studies did not find any differences in average telomere length between somatic tissues of reproductive and sterile female castes in ants and honeybees (Jemielity et al., 2007; Korandova and Frydrychova, 2016). However, it must be pointed out that both studies used for the telomere length assessments relatively young queens with the high potential to live much longer, and telomere length in senescent queens of the tested species remains elusive. Nevertheless, our data on telomerase activity in somatic tissues of *B. terrestris* are in agreement with our original hypothesis that lifespan in *B. terrestris* is regulated in a different way than in advanced social insects, and lifespan differences between *B. terrestris* workers and queens are rather enabled by the long diapause period.

According to the antagonistic pleiotropy theory of ageing, natural selection favors the genes that are beneficial early in life, such as genes of nutrient-sensing pathways, even though they are detrimental at later life stages and promote ageing (Blagosklonny, 2010; Williams, 1957). One such pathway is the gero-promoting TOR pathway (Antikainen et al., 2017; Hay, 2011; Stanfel et al., 2009; Tia et al., 2018), a conserved kinase, regulating cell growth and metabolism. The TOR pathway is activated by growth factors, nutrients, and insulin or other hormones, and antagonized by gero-suppressing factors, such as the FOXO transcription factor (Blagosklonny, 2010; Hay, 2011). Suppression of the TOR pathway or dietary restriction in adult age results in lifespan extension across vertebrate and invertebrate species (Blagosklonny, 2010). One of the well-known functions of TOR is its role in regulation of protein synthesis, where TOR activates two key targets, the ribosomal subunit SK6 and the eukaryotic initiation factor 4E-binding protein (4EBP). Upon its activation, 4EBP releases the eukaryotic translation initiation factor 4E, which is a key regulator of mRNA translation initiation (reviewed in Markaki and Tavernakakis, 2013). Protein intake has been shown as a crucial trigger for the

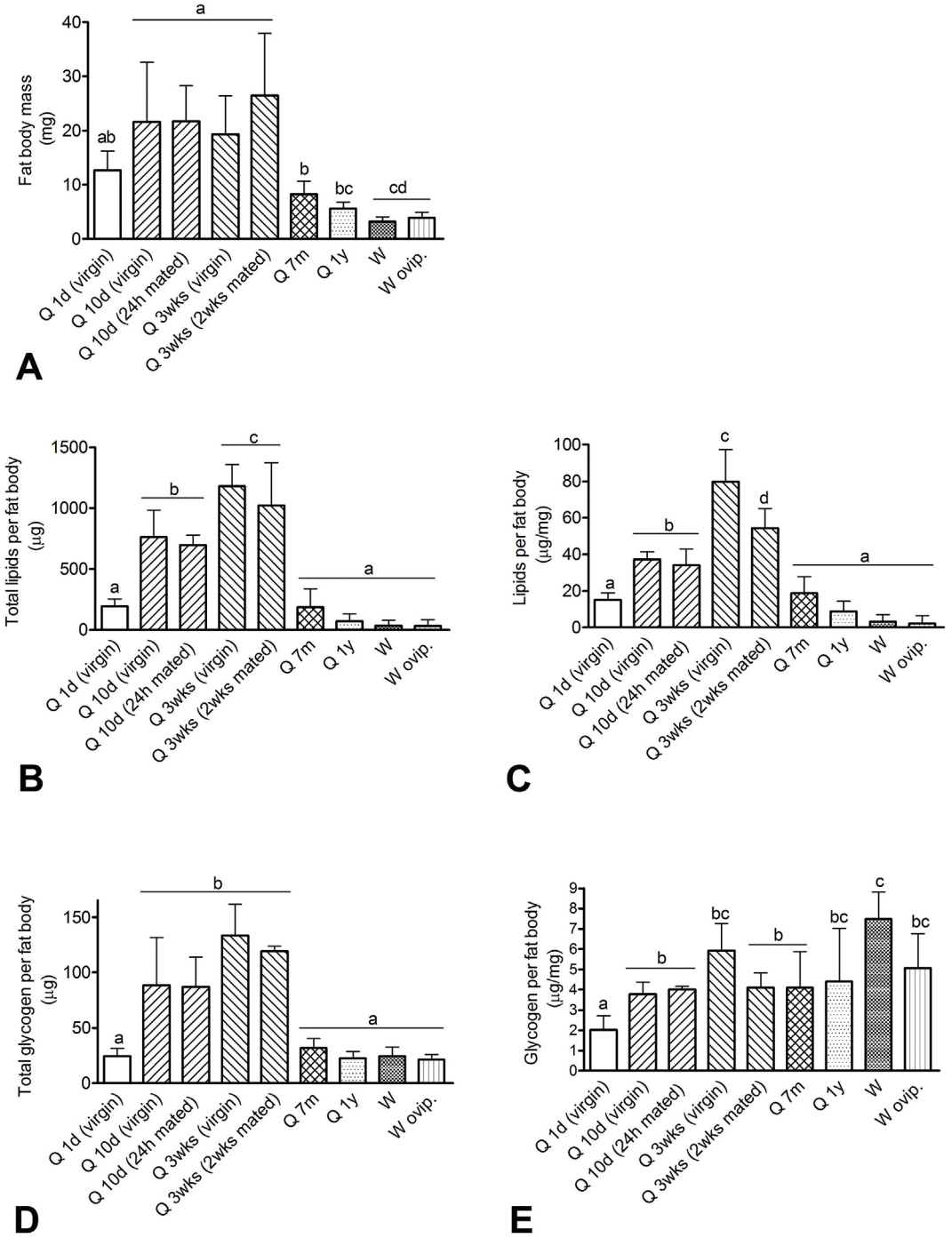



**Fig. 6.** The fat body mass and energy reserves in the fat body. The fat body mass **(A)**, total lipid amounts **(B)**, lipid concentrations **(C)**, total glycogen amounts **(D)**, and glycogen concentrations **(E)** were quantified in Q1d (virgin) – 1-day-old virgin queens; Q 10d (virgin) – 10-day-old virgin queens; Q 10d (24 h mated) – 10-day-old queens that were 24 h after mating; Q 3wks (2wks mated) – 3-week-old queens that were two weeks after mating; Q 7m – 7-month-old queens; Q 1year – 1-year-old queens; W ovip. – ovipositing workers; W – workers. Statistical significance was determined using One-way ANOVA and Tukey's post-hoc tests ($p < 5\%$, indicated by different letters above columns); n = 5. The bars in graphs represent the mean ± SD.

initiation of egg development in numerous insects requiring protein-rich diet, and TOR pathway serves as a nutritional control in female reproduction of such insects. SK6 activation mediated by TOR is involved in the regulation of the Vg production, and RNAi-mediated TOR silencing results in decreased Vg expression or severely affects egg production. Additionally, control of insect reproduction via TOR pathway is mediated by involvement of TOR in regulation of synthesis and secretion of JH and ecdysone (reviewed in Smykal and Raikhel, 2015). The TOR/FOXO transcript reversal we observed for 1-day-old and 1-year-old queens in their fat bodies indicates a high rate of metabolic stress in 1-year-old queens, which might be simply linked to reproductive status of the queens. In such case, however, it remains to be explained why similarly elevated levels of TOR transcripts were not observed in ovipositing workers. On the other hand, it needs to be also pointed out that a high metabolic rate is a feature of senescent cells (Nacarelli and Sell, 2017), and therefore, we can still ask whether the TOR/FOXO switch might be the senescence-related change. Nevertheless, lack of more detailed data makes it difficult to solve.

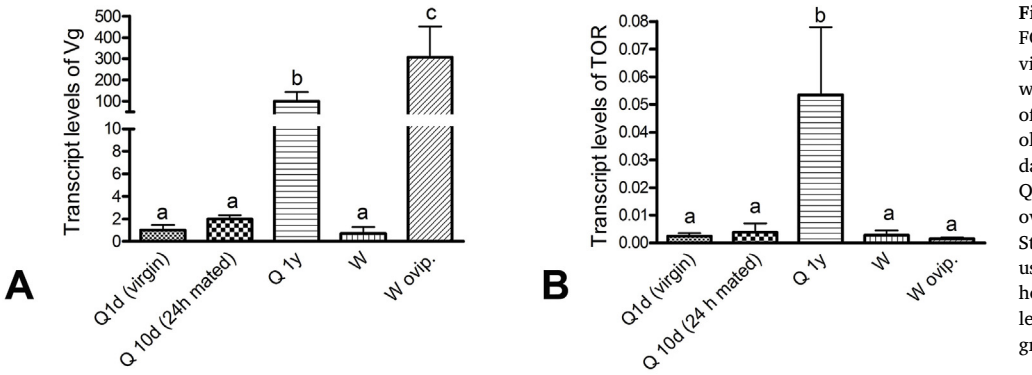
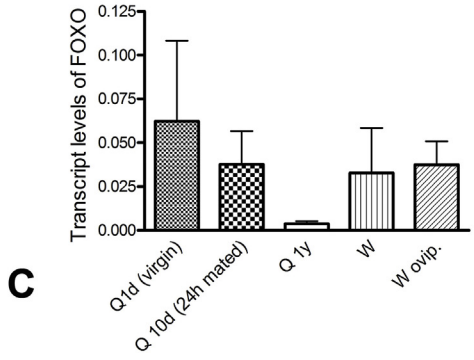



**Fig. 7.** Transcript levels of Vg, TOR, and FOXO in the fat bodies. Transcript levels of vitellogenin **(A)**, TOR **(B)** and FOXO **(C)** were evaluated relative to transcript levels of *elongation factor-1*. Q1d (virgin) – 1-day-old virgin queens; Q 10d (24 h mated) – 10-day-old queens that were 24 h after mating; Q 1year – 1-year-old queens; W ovip. – ovipositing workers; W – workers. Statistical significance was determined using One-way ANOVA and Tukey's post-hoc tests ($p < 5\%$, indicated by different letters above columns); $n = 5$. The bars in graphs represent the mean ± SD.



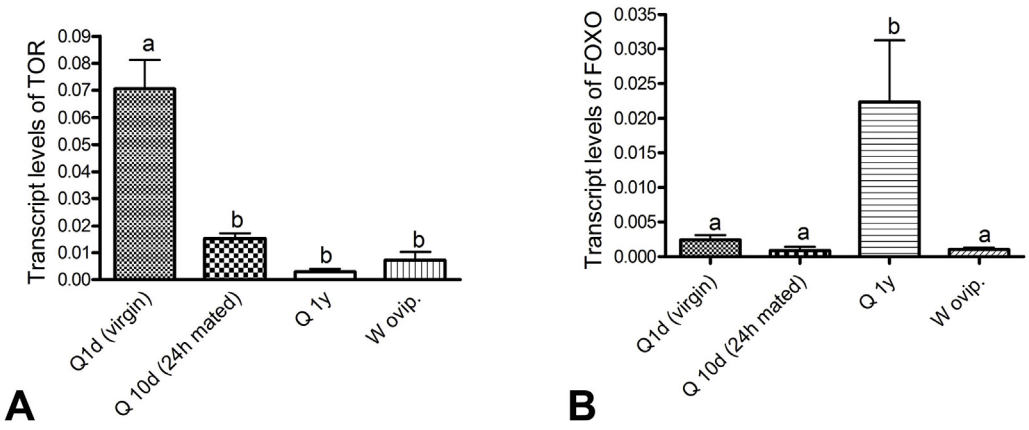




**Fig. 8.** Transcript levels of TOR and FOXO in ovaries. Transcript levels of TOR **(A)** and FOXO **(B)** were evaluated relative to transcript levels of *elongation factor-1*. Q1d (virgin) – 1-day-old virgin queens; Q 10d (24 h mated) – 10-day-old queens that were 24 h after mating; Q 1year – 1-year-old queens; W ovip. – ovipositing workers; W – workers. Statistical significance was determined using One-way ANOVA and Tukey's post-hoc tests ($p < 5\%$, indicated by different letters above columns); $n = 5$. The bars in graphs represent the mean ± SD.

In the fission yeast *Schizosaccharomyces pombe*, the loss of TOR1 leads to over-elongation of telomeres and chromatin changes, suggesting a possible link between nutrient sensing and cellular mechanisms that regulate chromatin structure and telomere maintenance (Schonbrun et al., 2009). Data about the role of the TOR pathway in telomere length maintenance in higher Eukaryotes are not available. In queens we found a negative correlation between telomerase activity/telomere length and transcript levels of TOR, complemented by a positive correlation between telomerase activity/telomere length and transcript levels of FOXO. However, to draw a conclusion that telomerase regulation is closely related to the TOR and FOXO pathways, or vice versa, or to explain the TOR/FOXO expression switch, more detailed experiments, such as RNA interference assays, need to be conducted. One of the TOR knock-down effects is reduced transcript levels of Vg, which plays a role not only in reproduction as a yolk precursor, but also with caste-dependent lifespan differences in honeybees, in antioxidant and anti-stress defense, and immune reactions (Guidugli et al., 2005; Havukainen et al., 2013; Kodrík et al., 2019; Münch et al., 2015). In honeybees, levels of Vg are elevated by protein-rich diets, and importantly, a knock-down of Vg has no effect on TOR transcript levels or queen development (Patel et al., 2007). In contrast to TOR and FOXO, we found no apparent correlation between changes in Vg transcript levels and telomerase activity/telomere length.

To conclude, apart from the upregulation of telomerase activity in embryos and gonads, which was expected, telomerase activity was enhanced in the fat bodies of young pre-diapause queens, and associated with increased DNA synthesis. We speculate that the upregulation of telomerase activity and DNA synthesis in the queens are essential for rapid intensification of metabolic activity in the fat body to build up a sufficient energy reserve prior to diapause. Data support our hypothesis that the lifespan differences between *B. terrestris* workers and queens are simply related to the long diapause period. Telomerase activity might be in a direct correlation with the mating process as well as with nutrient-sensing pathways; however, more detailed studies must be conducted to verify these relationships or uncover more hidden insights.

## Funding

This study was supported by grant no. 18-21200S of the Czech Science Foundation, by grant no. LTAUSA17116 Inter Action from Ministry of Education of the Czech Republic, by Strategy AV21, Diversity of Life and Health of Ecosystems.

## Conflicts of interest

Authors declare no conflict of interest.

## Acknowledgement

The authors thank Helena. Štěrbová and Matilda Emily Freytag for their technical assistance.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibmb.2019.103241.

## References

Aamodt, R.M., 2009. Age-and caste-dependent decrease in expression of genes maintaining DNA and RNA quality and mitochondrial integrity in the honeybee wing muscle. Exp. Gerontol. 44, 586–593. https://doi.org/10.1016/j.exger.2009.06.004.

Alford, D.V., 1969a. A study of the hibernation of bumblebees (Hymenoptera: Bombidae) in Southern England. J. Anim. Ecol. 38, 149–170.

Alford, D.V., 1969b. Studies on the fat body of adult bumble bees. J. Apic. Res. 8, 37–48.

Antikainen, H., Driscoll, M., Haspel, G., Dobrowolski, R., 2017. TOR-mediated regulation of metabolism in aging. Aging Cell 16, 1219–1233. https://doi.org/10.1111/acel.12689.

Aubert, G., Lansdorp, P.M., 2008. Telomeres and aging. Physiol. Rev. 88, 557–579. https://doi.org/10.1152/physrev.00026.2007.

Bens, M., Szafranski, K., Holtze, S., Sahm, A., Groth, M., Kestler, H.A., Hildebrandt, T.B., Platzer, M., 2018. Naked mole-rat transcriptome signatures of socially suppressed sexual maturation and links of reproduction to aging. BMC Biol. 16, 1–13. https://doi.org/10.1186/s12915-018-0546-z.

Blagosklonny, M.V., 2010. Revisiting the antagonistic pleiotropy theory of aging: TOR-driven program and quasi-program. Cell Cycle 9, 3171–3176. https://doi.org/10.4161/cc.9.16.13120.

Bloch, G., 1999. Regulation of queen-worker conflict in bumble-bee (Bombus terrestris) colonies. Proc. R. Soc. Lond. 266, 2465–2469. https://doi.org/10.1098/rspb.1999.0947.

Bloch, G., Hefetz, A., 1999. Regulation of reproduction by dominant workes in bumblebee. Behav. Ecol. Sociobiol. 45, 125–135.

Carey, J.R., 2001. Demographic mechanisms for the evolution of long life in social insects. Exp. Gerontol. 36, 713–722. https://doi.org/10.1016/S0531-5565(00)00237-0.

Chan, S.W., Blackburn, E.H., 2002. New ways not to make ends meet: telomerase, DNA damage proteins and heterochromatin. Oncogene 21, 553–563. https://doi.org/10.1038/sj/onc/1205082.

Chittka, A., Chittka, L., 2010. Epigenetics of royalty. PLoS Biol. 8, 8–11. https://doi.org/10.1371/journal.pbio.1000532.

Cong, Y., Wright, W.E., Shay, J.W., 2002. Human telomerase and its regulation. Microbiol. Mol. Biol. Rev. 66, 407–425. https://doi.org/10.1128/MMBR.66.3.407.

Corona, M., Hughes, K.A., Weaver, D.B., Robinson, G.E., 2005. Gene expression patterns associated with queen honey bee longevity. Mech. Ageing Dev. 126, 1230–1238. https://doi.org/10.1016/j.mad.2005.07.004.

Corona, M., Velarde, R.A., Remolina, S., Moran-lauter, A., Wang, Y., Hughes, K.A., Robinson, G.E., 2007. And queen honey bee longevity. Proc. Natl. Acad. Sci. 104, 7128–7133.

Davidovic, M., Sevo, G., Svorcan, P., Milosevic, D.P., Despotovic, N., Erceg, P., 2010. Old age as a privilege of the "selfish ones". Aging Dis 1, 139–146.

Denchi, E.L., 2009. Give me a break: how telomeres suppress the DNA damage response. DNA Repair (Amst) 8, 1118–1126. https://doi.org/10.1016/j.dnarep.2009.04.013.

Engels, W., 1990. Social Insects: an Evolutionary Approach to Castes and Reproduction. Springer-Verlag, New York.

Flores, I., Blasco, M.A., 2010. The role of telomeres and telomerase in stem cell aging. FEBS Lett. 584, 3826–3830. https://doi.org/10.1016/j.febslet.2010.07.042.

Folch, J., Lees, M., Sloane Stanley, G., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509. https://doi.org/10.1016/j.ultrasmedbio.2011.03.005.

Goldsworthy, G.J., Kodrík, D., Comley, R., Lightfoot, M., 2002. A quantitative study of adipokinetic hormone of the firebug, *Pyrrhocoris apterus*. J. Insect Physiol. 48, 1103–1109.

Goulson, 2010. Bumblebees: Behaviour, Ecology, and Conservation. Oxford University Press.

Guidugli, K.R., Nascimento, A.M., Amdam, G.V., Barchuk, A.R., Simo, L.P., 2005. Vitellogenin regulates hormonal dynamics in the worker caste of eusocial insect. FEBS Lett. 579, 4961–4965. https://doi.org/10.1016/j.febslet.2005.07.085.

Havukainen, H., Munch, D., Baumann, A., Zhong, S., Halskau, O., Krogsgaard, M., Amdam, G.V., 2013. Vitellogenin recognizes cell damage through membrane binding and shields living cells from reactive oxygen. J. Biol. Chem. 288, 28369–28381. https://doi.org/10.1074/jbc.M113.465021.

Hay, N., 2011. Interplay between FOXO, TOR, and Akt. Biochim. Biophys. Acta 1813, 1965–1970. https://doi.org/10.1016/j.bbamcr.2011.03.013.

He, X.J., Zhou, L. Bin, Pan, Q.Z., Barron, A.B., Yan, W.Y., Zeng, Z.J., 2017. Making a queen: an epigenetic analysis of the robustness of the honeybee (Apis mellifera) queen developmental pathway. Mol. Ecol. 26, 1598–1607. https://doi.org/10.1111/mec.13990.

Heidinger, B.J., Blount, J.D., Boner, W., Grif, K., Metcalfe, N.B., Monaghan, P., 2011. Telomere length in early life predicts lifespan. Proc. Natl. Acad. Sci. 109, 1–6. https://doi.org/10.1073/pnas.1113306109.

Herb, B.R., Wolschin, F., Hansen, K.D., J, A.M., Langmead, B., Irizarry, R., Amdam, G.V., Feinberg, A.P., 2012. Reversible switching between epigenetic states in honeybee behavioral subcastes. Nat. Neurosci. 15, 1371–1373. https://doi.org/10.1038/nn.3218.

Holm, S.V., 1972. Weight and life length of hibernating bumblebee queens (Hymenoptera: Bombidae) under controlled conditions. Entomol. Scand. 3, 313–320.

Jedlicka, P., Ernst, U.R., Votavová, A., Hanus, R., Valterová, I., 2016. Gene expression dynamics in major endocrine regulatory pathways along the transition from solitary to social life in a bumblebee, Bombus terrestris. Front. Physiol. 7, 1–20. https://doi.org/10.3389/fphys.2016.00574.

Jemielity, S., Chapuisat, M., Parker, J.D., Keller, L., 2005. Long live the queen : studying aging in social insects. Age (Omaha). 27, 241–248. https://doi.org/10.1007/s11357-005-2916-z.

Jemielity, S., Kimura, M., Parker, K.M., Parker, J.D., Cao, X., Aviv, A., Keller, L., 2007. Short telomeres in shortlived males: what are the molecular and evolutionary causes? Aging Cell 6, 225–233.

Jin, K., 2010. Modern biological theories of aging. Aging Dis 1, 72–74. https://doi.org/10.1016/j.bbi.2008.05.010.

Kirkwood, T.B.L., Holliday, R., 1979. The evolution of ageing and longevity. Proc. R. Soc. Lond. Ser. B Biol. Sci. 205, 531–546.

Kodrík, D., Ibrahim, E., Gautam, U., Čapková Frydrychová, R., Bednářová, A., Krištůfek, V., Jedlicka, P., 2019. Changes in vitellogenin expression caused by nematodal and fungal infections in insects. J. Exp. Biol. 222.

Korandova, M., Frydrychova, R.C., 2016. Activity of telomerase and telomeric length in *Apis mellifera*. Chromosoma 125, 405–411. https://doi.org/10.1007/s00412-015-0547-4.

Korandová, M., Krůček, T., Szakosová, K., Kodrík, D., Kühnlein, R.P., Tomášková, J., Čapková Frydrychová, R., 2018. Chronic low-dose pro-oxidant treatment stimulates transcriptional activity of telomeric retroelements and increases telomere length in Drosophila. J. Insect Physiol. 104, 1–8. https://doi.org/10.1016/j.jinsphys.2017.11.002.

Korandová, M., Krůček, T., Vrbová, K., Frydrychová, R.C., 2014. Distribution of TTAGG-specific telomerase activity in insects. Chromosome Res. 22, 495–503.

Kostal, V., Simek, P., 1998. Changes in fatty acid composition of phospholipids and triacylglycerols after cold-acclimation of an aestivating insect prepupa. J. Comp. Physiol. 168, 453–460. https://doi.org/10.1007/s003600050165.

Kucharski, R., Maleszka, J., Foret, S., Maleszka, R., 2013. Nutritional control of reproductive status in honeybees via DNA methylation. Science (80-. ) 319, 1827–1831. https://doi.org/10.1126/science.1153069.

Lee, H.O., Davidson, J.M., Duronio, R.J., 2009. Endoreplication: polyploidy with purpose. Genes Dev. 223, 2461–2477. https://doi.org/10.1101/gad.1829209.results.

Lin, N., Michener, C.D., 1972. Evolution of sociality in insects. Q. Rev. Biol. 47, 131–159.

Lopez-Vaamonde, C., Raine, N.E., Koning, J.W., Brown, R.M., Pereboom, J.J.M., Ings, T.C., Ramos-Rodriguez, O., Jordan, W.C., Bourke, A.F.G., 2009. Lifetime reproductive success and longevity of queens in an annual social insect. J. Evol. Biol. 22, 983–996. https://doi.org/10.1111/j.1420-9101.2009.01706.x.

Lucas, E.R., Keller, L., 2014. Ageing and somatic maintenance in social insects. Curr. Opin. Insect Sci. 5, 31–36. https://doi.org/10.1016/j.cois.2014.09.009.

Markaki, M., Tavernarakis, N., 2013. Metabolic control by target of rapamycin and Autophagy during ageing – a mini-review. Gerontology 59, 340–348.

Martins, G.F., Ramalho-Ortigão, J.M., 2012. Oenocytes in insects. Invertebr. Surviv. J. 9, 139–152.

Mason, J.M., Randall, T.A., Frydrychova, R.C., 2016. Telomerase lost ? Chromosoma 125, 65–73. https://doi.org/10.1007/s00412-015-0528-7.

Milewski, L.A.K., 2010. The Evolution of ageing. Biosci. Horizons 3, 77–84. https://doi.org/10.2307/1930748.

Münch, D., Ihle, K.E., Salmela, H., Amdam, G.V., 2015. Vitellogenin in the honey bee brain: Atypical localization of a reproductive protein that promotes longevity. Exp. Gerontol. 71, 103–108. https://doi.org/10.1016/j.exger.2015.08.001.

Nacarelli, T., Sell, C., 2017. Targeting metabolism in cellular senescence, a role for intervention. Mol. Cell. Endocrinol. 455, 83e92.

Nowak, M.A., 2010. Evolution of eusociality. Nature 466, 1057–1062. https://doi.org/10.1038/nature09205.

Patel, A., Fondrk, M.K., Kaftanoglu, O., Emore, C., Hunt, G., Frederick, K., Amdam, G.V., 2007. The making of a queen: TOR pathway is a key player in diphenic caste development. PLoS One 1–7. https://doi.org/10.1371/journal.pone.0000509.

Rodier, F., Campisi, J., Bhaumik, D., 2007. Two faces of p53: aging and tumor suppression. Nucleic Acids Res. 35, 7475–7484. https://doi.org/10.1093/nar/gkm744.

Schonbrun, M., Laor, D., Lopez-Maury, L., Bahler, J., Kupiec, M., Weisman, R., 2009. TOR Complex 2 controls gene silencing, telomere length maintenance, and survival under DNA-damaging conditions. Mol. Cell. Biol. 29, 4584–4594. https://doi.org/10.1128/MCB.01879-08.

Sergiev, P.V., Dontsova, O.A., Berezkin, G.V., 2015. Theories of aging: an ever-evolving field. Acta Naturae 7, 9–18.
Smeets, P., Duchateau, M.J., 2003. Longevity of *Bombus terrestris* workers (Hymenoptera: Apidae) in relation to pollen availability, in the absence of foraging. Apidologie 34, 333–337. https://doi.org/10.1051/apido.
Smykal, V., Raikhel, A.S., 2015. Nutritional control of insect reproduction. Curr Opin Insect Sci 11, 31–38.
Socha, R., Kodrík, D., Šimek, P., Patočková, M., 2004. The kind of AKH-mobilized energy substrates in insects can be predicted without a knowledge of the hormone structure. Eur. J. Entomol. 101, 1210–5759.
Stanfel, M.N., Shamieh, L.S., Kaeberlein, M., K, K.B., 2009. The TOR pathway comes of age. Biochim. Biophys. Acta 1790, 1067–1074. https://doi.org/10.1038/jid.2014.371.
Stoscheck, C.M., 1990. Quantitation of protein. Methods Enzymol. 182, 50–68.
Szathmáry, E., Smith, J.M., 1995. The major evolutionary transitions. Nature 374, 227–232.
Tia, N., Singh, A.K., Pandey, P., Azad, C.S., Chaudhary, P., Gambhir, I.S., 2018. Role of Forkhead Box O (FOXO) transcription factor in aging and diseases. Gene 648, 97–105. https://doi.org/10.1016/j.gene.2018.01.051.
Votavová, A., Tomčala, A., Kofroňová, E., Kudzejová, M., Šobotník, J., Jiroš, P., Komzáková, O., Valterová, I., 2015. Seasonal dynamics in the chemistry and structure of the fat bodies of bumblebee queens. PLoS One 10, 1–14. https://doi.org/10.1371/journal.pone.0142261.
West, S.A., Fisher, R.M., Gardner, A., Kiers, E.T., 2015. Major evolutionary transitions in individuality. Proc. Natl. Acad. Sci. 112, 10112–10119. https://doi.org/10.1073/pnas.1421402112.
Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution (N. Y). 11, 398. https://doi.org/10.2307/2406060.
Wright, W.E., Piatyszek, M.A., Rainey, W.E., Byrd, W., Shay, J.W., 1996. Telomerase activity in human germline and embryonic tissues and cells. Dev. Genet. 18, 173–179.
Zöllner, N., Kirsch, K., 1962. Colorimetric method for determination of total lipids. J. Exp. Med. 135, 545–550.