School of Doctoral Studies in Biological Sciences

University of South Bohemia in České Budějovice
Faculty of Science

Telomere length compensation mechanisms as players in longevity and stress adaptation of insects

Ph.D. Thesis

RNDr. Michala Korandová

Major supervisor: RNDr. Radmila Čapková Frydrychová, Ph.D.

Institute of Entomology, Biology Centre of the ASCR, České Budějovice

Supervisor from University: prof. RNDr. Dalibor Kodrík, CSc.

Faculty of Science, University of South Bohemia in České Budějovice

Institute of Entomology, Biology Centre of the ASCR, České Budějovice

České Budějovice 2017

This thesis should be cited as:

Korandova, M (2017). Telomere length compensation mechanisms as players in longevity and stress adaptation of insects. Ph.D. Thesis. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 123 pp.

Annotation

Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes that are important for genome stability and integrity. They are shortened with each cell cycle and during organismal aging. Although the most common telomere length compensation mechanism is the activity of a special reverse transcriptase, telomerase, in Drosophila telomeres are maintained by the retrotransposition of telomeric elements. In mammals, telomere length and telomerase activity can be influenced by lifestyle and the environmental conditions. This thesis is focused on activity of telomere length maintenance mechanism in insects in relation to aging and stress response.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 31. 10. 2017 .....

Michala Korandová

This thesis originated from a partnership of Faculty of Science, University of South Bohemia, and Institute of Entomology, Biology Centre CAS, supporting doctoral studies in the Physiology and Development Biology study program.

Financial support

This work was supported by the grant no. 14-07172S from the Grant Agency of the Czech Republic. Then by the Grant No. 052/2013/P and the grant no. 038/2014/P from the Grant Agency of the University of South Bohemia. We acknowledge the use of research infrastructure that has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 316304.

Acknowledgements [in Czech]

Na tomto místě bych ráda v první řadě poděkovala své školitelce RNDr. Radmile Čapkové Frydrychové, Ph.D., za cenné rady, ochotu, trpělivost a celkové odborné vedení této práce a za veškeré zkušenosti, které jsem mohla získat jak během magisterského, tak doktorského studia pod jejím vedením. Dále nesmím zapomenout na všechny spoluautory publikací a členy laboratoří prof. RNDr. Františka Marce, CSc. a prof. RNDr. Dalibora Kodríka, CSc. za přátelskou atmosféru. Dík patří také Ing. Václavu Krištůfkovi, Csc., který mi svým nadšením ukázal, že včela není životu nebezpečný organismus.

Chtěla bych dále poděkovat celé své rodině za jejich nesmírnou podporu po celou dobu studia, a že nikdy nepřestali věřit a doufat.

List of papers and author's contribution

The thesis is based on the following papers:

I. **Korandová M**, Krůček T, Vrbová K, Čapková Frydrychová R (2014). Distribution of TTAGG-specific telomerase activity in insects. Chromosome Research **22**: 495-503. (IF = 2.385).

Hereby I declare that Michala Korandová participated in this publication in a significant way, mainly by collecting samples, preparing and performing TRAP assay, cloning, evaluation of the results of Southern hybridization, and she participated in text preparation of manuscript.

RNDr. Radmila Čapková Frydrychová, Ph.D.

Corresponding author, team leader

II. **Korandová M**, Čapková Frydrychová R (2016). Telomerase activity and telomere length in *Apis mellifera*. Chromosoma **125**: 405-411. (IF = 4.414).

Hereby I declare that Michala Korandová participated in this publication in a significant way, mainly by collecting samples, preparing and performing TRAP assay, cloning, evaluation of the results, and she participated in text preparation of manuscript.

RNDr. Radmila Čapková Frydrychová, Ph.D.

Corresponding author, team leader

III. **Korandová M**, Krůček T, Szakosová K, Kodrík D, Kühnlein RP, Tomášková J, Čapková Frydrychová R (manuscript). Chronic low-dose pro-oxidant treatment stimulates transcriptional activity of telomeric retroelements and increases telomere length in *Drosophila*

Hereby I declare that Michala Korandová participated in this publication in a significant way, mainly by collecting samples, preparing and performing quantitative real-time PCR, statistical evaluation of the results, and she participated in text preparation of manuscript.

RNDr. Radmila Čapková Frydrychová, Ph.D.

Corresponding author, team leader

CONTENT

1. GENERAL INTRODUCTION

Telomeres are special nucleoprotein structures at the ends of eukaryotic chromosomes. Telomeres are important for genome stability and integrity as they allow cells to distinguish natural chromosome ends from ends generated by double-strand breaks, they protect chromosome ends from chromosome fusions and action of nucleases (Chan & Blackburn 2002; von Zglinicki 2002; Cenci et al. 2005; Denchi 2009). Also, telomeres compensate losses of terminal DNA, known as telomere shortening, caused by incomplete DNA replication or oxidative stress. There are several ways how to compensate telomere shortening, but the most common mechanism is the activity of a special reverse transcriptase, called telomerase. This enzyme binds to chromosome ends where repeatedly attaches short sequences of telomeric DNA. The activity of telomerase is linked to cells proliferation and the absence of telomerase activity is a marker of cell senescence and aging. Telomerase insufficiency is associated with conditions and diseases that can shorten organismal lifespan (Lin et al. 2012; Lin et al. 2008). Role of telomerase is hypothesised in extended longevity of reproductive individuals of social insects, such as honey bees (Korandova & Frydrychova 2016).

1.1. Common structure of telomeric DNA sequence

The most common DNA structure of telomeres is a short repetitive sequence (5′-$T_xA_yG_z$-3′) formed by telomerase. The 3’ end of telomeric DNA creates single-strand overhang that folds back and invades the duplex region to form a specialized displacement loop called a t-loop. The formation of t-loop presumably helps shield telomere ends from recognition as double-stranded breaks (Makarov et al. 1997; Williamson et al. 1989). Although there is some conservancy in telomere sequence composition across phyla, telomeric sequence can be different in different taxonomic groups (Zakian 1995; Makarov et al. 1997; Snustad & Simmons 2000; Capkova Frydrychova & Mason 2013). For example, general telomeric sequence of fungi and Amoebozoa is $(T_2AG_3)n$, while the $(T_3AG_3)n$ telomeric sequence is found in most plants and green algae, and the (TTAGG)n is telomeric sequence in all vertebrates (Meyne et al. 1989; Guo & Allen 1997; Viturri et al. 2000; Chan & Blackburn 2002; Fajkus et al. 2005; Capkova Frydrychova & Mason 2013).

1.1.1. Protein structure of telomeres

The telomere protective function against chromosomal fusions and genomic instability is primarily given by a multiprotein structure at chromosome ends called a telomere cap. Telomere cap dysfunction results in chromosomal fusions, followed by chromosomal breakage, loss of genetic material, genomic instability, and eventually apoptosis (Linger & Price 2010). In humans, telomere cap consists of shelterin, which is a multiprotein complex formed solely on telomeric DNA sequence, and non-shelterin telomeric proteins, which bind to both telomeric and non-telomeric DNA and may perform various genomic functions. For instance, non-shelterin proteins ATM, ATR or KU70/80 are involved in DNA damage response mechanism (Cenci et al. 2005). Shelterin consists of six proteins - TRF1, TRF2, TIN2, TPP1, POT1 and Rap1. The TRF1 (telomeric repeat-binding factor 1) and TRF2 (telomeric repeat-binding factor 2) proteins bind to double-stranded telomeric DNA and recruit TIN2 (TRF1- interacting nuclear factor 2) and TPP1 (tripeptidyl peptidase). The TPP1 and TIN2 make a bridge between the TRF proteins and G-overhang-binding protein POT1 (Smogorzewska & de Lange 2004; Raffa et al. 2005; Raffa et al. 2011). In vertebrates telomere cap formation requires a terminal DNA array of specific sequence and of satisfactory length (reviewed in Mason & Capkova Frydrychova 2013).

Instead of shelterin Drosophila telomeres contain tetrameric complex called terminin, composed of HOAP, Moi, Ver and HipHop proteins (Cenci et al. 2005, Raffa et al. 2005, Mason et al. 2008). Although shelterin and terminin substantially differ, a comparison between vertebrate shelterin proteins and Drosophila non-terminin proteins show high homology (Capkova Frydrychova & Mason 2013; Cenci et al. 2005).Terminin significantly differs from shelterin in terms of its binding to DNA as terminin formation does not require specific DNA sequence and can be formed on any chromosome end. Therefore, the loss of telomeric sequence in Drosophila does not necessarily lead to chromosomal fusions and subsequent genomic instability (Capkova Frydrychova & Mason 2013).

1.2. Telomerase as the most common telomere elongation mechanism

Because of the inability of conventional DNA polymerase to fully synthesize ends of linear DNA molecule telomeres are progressively shortened during each cell cycle (Hayflick &

Moorhead 1961; Olovnikov 1973; Blackburn 1991). There are several ways how to solve telomere shortening, but the most common mechanism of telomere length compensation is the activity of telomerase. Telomerase is a ribonucleoprotein complex consisted of RNA subunit (called TER, Telomerase RNA domain), as a template for telomeric sequence, and reverse transcriptase (TERT, Telomerase Reverse Transcriptase), which uses the RNA template to synthesize new telomeric repeats onto chromosome ends (Prowse & Greider 1995; Blasco et al. 1997; Zhou et al. 2014). The activity of telomerase consists of several steps (Fig. 1): *(1)* telomerase binds to 3´ end of chromosome, which is used as DNA primers for subsequent DNA synthesis, *(2)* the RNA template is transcribed into new telomeric repeat that is attached to the 3' end (Wyatt et al. 2010), *(3)* telomerase is translocated to the 3' end of the new telomeric repeat, and the whole process is repeated. The 5' end of DNA is synthesized by conventional polymerase α and primase (Wyatt et al. 2010; Cooper 2000).

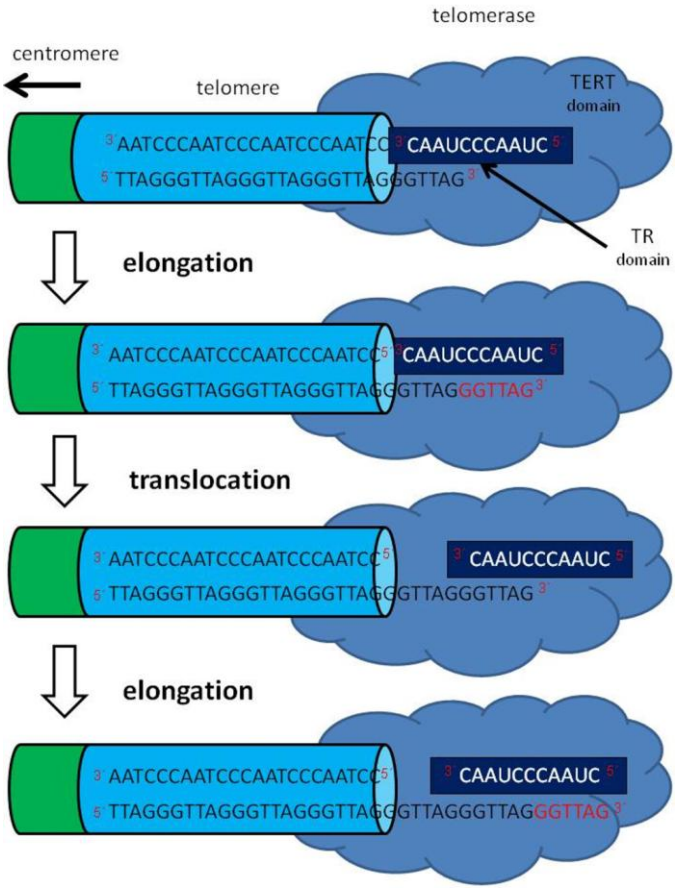

Figure 1: The process of telomere elongation by telomerase. Telomerase binds to chromosome end to 3´ end. The 3´ end is elongated by attaching of newly synthesised telomeric repeat. Then, telomerase moves to 3´ end of newly attached repeat.

Telomerase is generally expressed in proliferative cells. In humans, the highest telomerase activity is recorded during early embryonic development, and during subsequent development overall activity of telomerase becomes down-regulated. Although most somatic cells in adult organisms show cell senescence status and absence of telomerase or its very low activity, there are some somatic cells in adults showing high proliferation rate together with high telomerase activity. These cells are, for instance, cells of bone marrow, skin, gastrointestinal tract, testes or activated lymphocytes and cancer (Kim et al. 1994; Harley & Villeponteau 1995; Yasumoto et al. 1996; Shay & Bacchetti 1997; Yui et al. 1998; Liu et al. 1999; Forsyth et al. 2002; Geserick & Blasco 2006). The activity of telomerase maintains different telomere length in various parts of human body, and the longest telomeres are found in testes, skin, small intestine, cornea or brain (Flores et al. 2006).

The correlation between telomerase and cell proliferation activity might be a common phenomenon as was also found in higher plants (in *Melandrium album*), where upregulation of telomerase was observed in germinating seedlings and root tips, in contrast to leaves or quiescent seeds showing low and no telomerase activity, respectively (Riha et al. 1998). Surprisingly, high levels of telomerase activity was observed in all tested organs in a lobster (*Homarus americanus*) (Klapper et al. 1998). This might be explained by a life-long high proliferative capacity in differentiated tissues together with high capability of tissue regeneration in adult lobsters (Klapper et al. 1998). However, a tissue-independent activity of telomerase was also observed in *Periplaneta americana* (a cockroach) as a representative of hemimetabolous insects (Sasaki & Fujiwara 2000).

1.3. Telomere sequence and activity of telomerase in insects

Although the (TTAGG)n sequence is the ancestral telomeric sequence in Arthropod (Sahara et al. 1999) and the most common telomere sequence in insects, its frequent losses occurred during insect evolution (Frydrychova & Marec 2002; Frydrychova et al. 2004; Vítková et al. 2005). While the (TTAGG)n sequence is well conserved in neopteran orders such as Hymenoptera, Lepidoptera, Trichoptera or Orthoptera, it was disappeared in primitive Paleoptera. Although the actual telomeric sequence in insect orders that display the absence of (TTAGG)n motif remains mostly unknown, frequent switch points between the TTAGG

presence and absence, particularly in the order Coleoptera (beetles) (Frydrychova & Marec 2002), indicate that evolution of telomeres might be highly dynamic.

The TTAGG sequence was designated as the telomeric sequence in numerous insect species as was found to be present at their telomeres (Frydrychova et al. 2004; Frydrychova & Marec 2002; Traut et al. 2007), and it has been generally accepted that the sequence is the functional telomeric sequence maintained by telomerase. However, this assumption may not be true, at least in some cases, as revealed by situation in *Bombyx mori* (Lepidoptera), where telomerase is not active regardless of the (TTAGG)n presence at telomeres. It is speculated that TTAGG sequence found at *B. mori* telomeres is non-functional relic of the preceding telomerase system, which has been replaced by alternative telomere maintenance mechanism (Fujiwara et al. 2005). There is an opinion that the telomeric cap is precisely the decisive factor that provides specific telomere extension and that telomerase is the first effective mechanism associated with this protein complex (Mason et al. 2016).

1.4. Alternative structure of telomeres

Although telomeres mostly consist of short telomeric repeats, formed by telomerase, there are several exceptions to this rule, such as telomeres of *Chironomus* species or *Anopheles gambiae*, where telomeres are maintained by gene conversion of long satellite sequences, or telomeric retroelements in Drosophila (Capkova Frydrychova & Mason 2013; Schubert et al. 1994; Pich et al. 1996; Pich & Schubert 1998; Fajkus et al. 1995). Drosophila telomere is the most studied non-canonical telomere structure at all (Mason et al. 2008; Capkova Frydrychova et al. 2009).

1.4.1. Drosophila telomeric retroelements

Telomeres of *Drosophila melanogaster* consist of multiple copies of three telomere specific non-LTR retroelements *HeT-A*, *TART* and *TAHRE* (abbreviated as HTT elements), which are present at telomeres at various numbers and order. The HTT elements compensate telomere losses by their retrotransposition, which is driven specifically to chromosome ends (Mason et al. 2008; Capkova Frydrychova et al. 2009). Based on sequence similarities

between the HTT elements it seems that the elements originated from one common ancestor (Shpiz et al. 2007).

The best studied telomere retroelement in Drosophila is *HeT-A*. This element is 6 kb long and has one open reading frame (ORF) encoding Gag protein (Biessmann & Mason 2003). The most noticeable feature of the element is its promoter situated at 3´ UTR of the element; thus, the *HeT-A* promoter controls the transcription of neighbouring 3´element (Pardue & DeBaryshe 2003). Since the transcription start is also located in the area of promoter, each transcript has a short section (approximately 60 bp) originating from the 5´neighbouring element. Another promoter is located at 5´ UTR of *HeT-A*, but it is weak and contributes to the total transcription only slightly. Since *HeT-A* element does not code own reverse transcriptase, its transposition is dependent on *TAR*T and *TAHRE* elements (Danilevskaya et al. 1997; Danilevskaya et al. 1999; Pardue & DeBaryshe 2003).

TART is 12 kb non-LTR retrotransposon, which has two open reading frames (ORF1 and ORF2) (Capkova Frydrychova et al. 2009) encoding protein with endonuclease activity and reverse transcriptase (Casacuberta & Pardue 2005). The *TAHRE* element (Telomere-Associated and *HeT-A* Related Element) (Abad et al. 2004) has two open reading frames with sequence similarities to ORF(s) of TART (Shpiz et al. 2007).

1.4.2. Retrotransposition

The major mechanism of telomere elongation in Drosophila is retrotransposition of HTT elements. The mechanism of retrotransposition works in a similar way as found in usual non-telomeric retroelements (Fig. 2): *(1)* the transcription of retroelements, *(2)* the transcripts are driven out from nucleus through nuclear pores, *(3)* the transcripts are translated into Gag and reverse transcriptase, *(4)* Gag protein binds to the retroelement transcripts and the whole complex is transported back into cell nucleus. Then, the transcripts are attached to chromosome ends, where are converted to DNA using reverse transcriptase (George & Pardue 2003; Biessmann & Mason 2003). The HTT transcripts are targeted to chromosome ends independently on their DNA sequence (Traverse & Pardue 1988; Biessmann et al. 1990; Biessmann et al. 1992).

The activity of telomeric elements correlates with cell proliferation and it is primarily targeted to diploid cells, mostly found in gonads or cells of imaginal discs (George & Pardue 2003; Danilevskaya et al. 1999; Pardue & DeBaryshe 2003; Walter & Biessmann 2004).

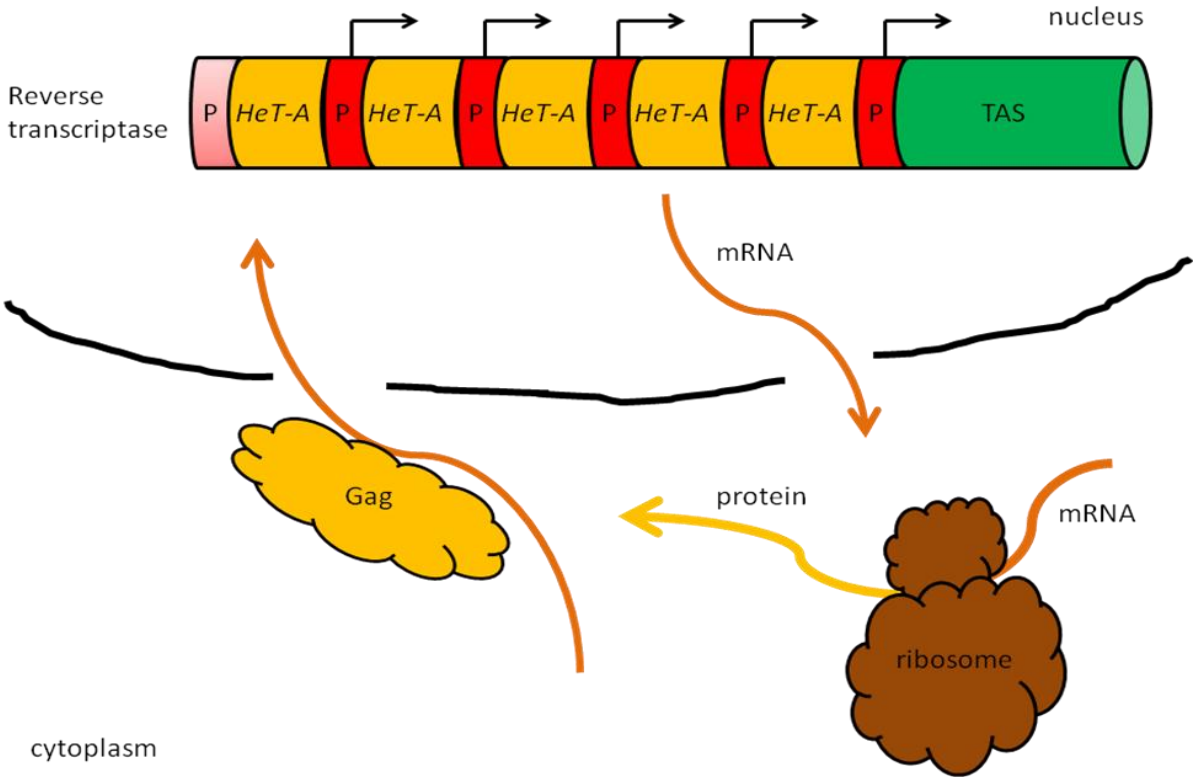

Figure 2: A scheme of telomeric retrotransposition in *Drosophila*. *HeT-A* is transcribed from promoter located at 3´ UTR region. The mRNA molecules go through nucleus pores to cytoplasm, where are translated into Gag protein and reverse transcriptase. Gag protein is bound to the retroelement transcripts, and this nucleoprotein complex is transported back into the nucleus. The mRNA molecules are attached to chromosome ends, where are transcribed into DNA using reverse transcriptase.

1.5. Telomere length is affected by oxidative stress and other stressors

Besides the incomplete DNA replication, telomeres are shortened by oxidative stress. Oxidative stress is defined as impaired equilibrium between production of free radicals and their consecutive elimination using antioxidant systems in cells (Sies 1997). It is assumed that the involvement of free radicals in telomere shortening rate is much greater than it is the incomplete replication effect (Oikawa et al. 2001). Although there are numerous exogenous

sources of free radicals, such as polluted environment, UV radiation or ionizing radiation (Ermak & Davis 2002; Abdollahi et al. 2004; Cheng et al. 2010), the main source of free radicals, particularly free oxygen radicals, such as superoxide anion or hydroxyl radical, is endogenous. Free oxygen radicals in organisms arise mainly from the respiratory chain in mitochondria (Chance et al. 1979; Grigolava et al. 1980; Ott et al. 2007), immune reactions (Cadenas & Sies 1998; Yasunari et al. 2002) or detoxication events using cytochrome P450 (Lewis et al. 1998).

Negative aspects of oxidative stress on cellular functions and organismal health are broadly known. Oxidative stress is associated with a damage of cellular components, which may lead to disruption of cell signalling and homeostasis, cell cycle arrest or even apoptosis (Valko et al. 2007). Also, there are numerous health problems that might be associated with oxidative stress, such as cancer, Parkinson diseases or premature aging (Griendling & FitzGerald 2003, Klaunig et al. 2010, Barnham et al. 2004, Maritim et al. 2003, Cadenas & Davies 2000). It is well known that oxidative stress has genotoxic effect; the most severe damage caused by free oxygen radicals in genome is found at telomeres. When compared to non-telomeric regions, a high amount of guanine makes telomeres particularly sensitive to oxidative damage. 8-Oxoguanine is one of the most common oxidative base modifications caused by oxidative stress in genome, and it leads to a mismatched pairing with adenine and subsequent G to T and C to A substitutions (Loft a Poulsen 1996). In humans, 8-Oxoguanine sites are primarily repaired by DNA glycosylase OGG1, which creates single-strand breaks as intermediates during DNA repair. However, a specific telomeric chromatin structure decreases efficiency of DNA repair mechanisms at telomeres leaving a high amount of single-strand breaks unrepaired. During a subsequent DNA replication, when the replication fork is formed, the telomeric region that is located distally to the single-strand break falls off (Oikawa & Kawanishi 1999; von Zglinicki 2002; Dizdaroglu et al. 2002; Houben et al. 2008; Nasir et al. 2014).

1.6. The attrition of telomeres as an indicator of biological age

The telomeric attrition is one of cellular mechanisms that are thought to be particularly involved in physiological and cellular processes underlying variation in lifespan between

different individuals. Telomere length may act as a marker of organismal age and health condition as numerous studies in vertebrates showed that both aging and various degenerative diseases are associated with progressive telomere shortening (Harman, 1956; Chen et al. 1998; von Zglinicki, 2000). Although it seems that length of telomeres may, at least to a certain extent, predict length of subsequent lifespan, it should be pointed out that variation of telomere length between individuals of the same age is not only due to genetic predisposition but also is caused by various environmental factors, which may greatly affect telomere length and telomerase activity (Entringer et al. 2011; Epel et al. 2004; Lin et al. 2012, Heidinger et al. 2012).

The telomere attrition in relation to stress acting was mostly studied in vertebrates, where, besides the effect of reactive oxygen species, telomere length has been found to be decreased as a result of psychological stress (Epel et al. 2004; Damjanovic et al. 2007; Parks et al. 2009; Epel et al. 2010; Entringer et al. 2011), or telomere loss might be accelerated by metabolically expensive activities such as reproduction, when presumably production of reactive oxygen species is enhanced (Heidinger et al. 2012; Kotrschal et al. 2007; Metcalfe & Alonso-Alvarez 2010). Stress effect on telomeres has been reported even in invertebrate species. In midges *Chironomus* heat-shock stress conditions led to transcriptional upregulation of satellite telomeric sequences (Martínez-Guitarte et al. 2008) or in yeast where the TLM (Telomere Length Maintenance) genes are influenced (Romano et al. 2013).

1.6.1. Aging

Aging is a gradual, time-related multifactorial process that leads to accumulation of cellular changes associated with a decrease of biological system functions, and resulting in a death of organism (Viña et al. 2007; Kirkwood 2005; Rose & Finch 1994). Although the nature of aging and underlying causes of aging process remain mysterious, several aging theories were postulated. Traditional theories of aging proposes that aging process is induced based on accidental and random damages in organism evolved by acting of endogenous and exogenous stress conditions. It includes the free radical theory proposing deleterious free radical reactions, which constantly take place in cells and tissues, as the major contributor of aging (Harman 1956; Harman 1972), or glycation theory (Suji & Sivakami 2004). Glycation

is produced by interaction between the amino groups of lysine and the aldehyde groups of glucose via a Schiff base reaction, yielding a wide range of advanced glycation end-products, which strongly affects a functionality of protein components of all biological systems (Gkogkolou & Böhm 2012). In contrast, modern biological theories of aging suggest that aging is a programmed process following a certain biological timetable associated with a sequential up- or down- regulation of certain genes, which includes hormonal regulation or gradual decline of immune system (reviewed in Kunlin 2010).

Numerous genes have been discovered to play a role in aging, such as genes involved in metabolic or endocrine signalling pathways (Korb 2016; Monroy Kuhn & Korb 2016). Number of studies showed that aging is hormonally regulated with a key role of the evolutionarily conserved insulin/IGF-1 signalling (IIS) pathway (Partridge et al. 2011) and insulin/TOR signalling pathway. TOR (Target of Rapamycin) kinase is a component of the molecular nutrient sensor pathway. In response to environmental cues, TOR signalling is activated by growth factors (such as IGF-I), insulin and other hormones and nutrients, like amino acids, glucose and fatty acids (Partridge et al. 2011). In adult life inhibition of the TOR pathway, for instance, by caloric restriction decelerates aging process, while a decrease or inactivation of TOR during a development stage of life suppresses growth or has lethal effects, respectively. TOR is one of antagonistically pleiotropic genes (also called gerogenes). The inactivation of gerogenes decreases fitness in early life, whereas promotes longevity in adult age (Blagosklonny 2010). Several gerosupressors, antagonizing the TOR pathway, has been reported, such as sirtuins, FOXO, or PTEN (Guarente 2007; Ortega-Molina & Serrano 2013; Martins et al. 2016).

1.6.2. Aging process at honey bees

Cost of reproduction is the conventional wisdom of a trade-off between reproduction and lifespan (Reznick 1985; Harshman & Zera 2007). Although the phenomenon is generally observed across phyla, it is contradicted in eusocial insect, which shows a strikingly prolonged lifespan in reproductive caste compared to non-reproductive (Partridge & Harvey 1985). A honeybee queen (*Apis mellifera*), the only reproductive female in a colony, can live about 60 times longer than honeybee workers (Flanders 1960). During summer adult lifespan

of honey bee worker is 4-6 weeks showing an age-dependent increase of mortality from low in nurses, which are young worker bees not yet ready to leave the hive and performing „house-hold“ type work, to high in foragers, which are bees leaving a hive to collect nectar and pollen (Johnson 2010). If the progression from nursing to foraging is prevented, the worker's lifespan extends to 10-19 weeks (Guidugli et al. 2005). The winter bees can live up to eight months and can only be outlived by the queens. Honey bee queen can survive for up to 8 years (Remolina & Hughes 2008; Page & Peng 2001). Although exact mechanisms are unclear the caste-specific differences in longevity in honey bees can be explained by different hormonal regulation in different honey bee castes.

One factor that plays a role in lifespan differences in honey bees is vitellogenin, a 118-kDa yolk protein precursor, which protects bees from oxidative and immune insults. The level of vitellogenin strongly drops during nurse/forage transition, it is enhanced in long-lived winter bees, and the highest vitellogenin level is found in queens (Havukainen et al. 2013). Vitellogenin inhibits production of juvenile hormone, which is the major endocrine stimulator of insect reproduction and a pro-aging factor in bees (Havukainen et al. 2013; Seehuss et al. 2006; Guidugli et al. 2005). Juvenile hormone as well as TOR seems to play in antagonistically pleiotropic fashion in bees. When compared to honey bee workers, the activity of both proteins are enhanced during the larval development of queen, perhaps resulting in the accelerating larval queen development and increased body size. In contrast, both proteins decline during the adult life of queen, which is conceivably associated with the prolonged longevity of honey bee queens (Corona et al. 2007).

Besides genetic factors, organismal longevity is influenced by environmental factors, such as nutrition or stress (Brodschneider & Crailsheim 2010). In honey bees, nectar or honey provide carbohydrates for energy, whereas pollen is the sole source of proteins essential for development (Buttstedt et al. 2014). Feeding royal jelly, a secretion from the hypopharyngeal glands of nurse bees, is also crucial for the switch from the worker to queen fate. Royal jelly is remarkably rich in proteins, lipids, carbohydrates, and many bioactive substances, and it plays a role in cell regeneration and anti-aging processes (Buttstedt et al. 2016). Royal jelly is the food given to all larvae during the first 2-3 days of their development. For queens, royal jelly is the exclusive food for their entire life (Buttstedt et al. 2014). The queen development is thus determined by high amount of well-balanced nutrients (Buttstedt et al. 2014; Buttstedt

et al. 2016), and it is regulated by a cross-talk among several signalling pathways involving ecdysteroids, juvenile hormone, vitellogenin, the epidermal growth factor (EGF) (Formesyn et al. 2014), the nutrient-sensing insulin/insulin-like growth factor 1 signalling (IIS) (Mutti et al. 2011; Wang et al. 2013), and target of rapamycin (TOR) pathways (Patel et al. 2007). To induce queen development, EGF activates a number of effectors leading to increased body size (e.g. TOR/S6K signalling). MAPK-dependent activation of ecdysteroid synthesis shortens the developmental time, and increased JH titter during the fourth larval instar induces ovarian development (Kamakura 2011).

Pollen is the only source of proteins at honeybees, and so far no adequate substitute of pollen has been found (Aronstein & Hayes 2004; Bilikova et al. 2013; Eremia et al. 2013). As an essential component for royal jelly production, pollen is critically important for nourishment of honey bee queens and larval development of honey bees, and therefore for brood production and existence of honey bee colony. Ingestion of pollen by nurse bees, which are the young bees that provide royal jelly to brood or queen, regulates lifespan of adult workers as the protein-rich diet improves nutritive status of bees, increases vitellogenin levels in adult workers, which in turn decreases levels of juvenile hormone (Corona et al. 2007). Low pollen resources and impaired quality of pollen are considered as one cause of serious decline of honey bee population that is registered world-wide in last two decades (vanEngelsdorp et al. 2009; vanEngelsdorp & Meixner 2010).

2. OUTLINE AND AIMS OF THE RESEARCH

1) Telomerase activity in holo- and hemimetabolous insects

Research on a wide range of organisms suggests that telomerase is, if not almost universal, the most frequent mechanism how to compensate telomere length (Gomes et al. 2011; Chan & Blackburn 2002; McEachern & Blackburn 1994). The (TTAGG)n, which is the ancestral arthropodal telomeric sequence, has been found at telomeres in most of the tested insect orders, however, there are numerous insect species that showed its absence. Frequent losses of the TTAGG sequence in insect seem to be rather suspicious and, regarding *B. mori* where the TTAGG presence was proved despite the absence of telomerase (Sasaki & Fujiwara 2000), it arises a question if the (TTAGG)n sequence is functional telomeric motif in insects maintained by telomerase.

The activity of telomere length compensation mechanisms have been repeatedly shown to be up-regulated in proliferative tissues, which was observed not only in numerous vertebrate models (reviewed in Gomes et al. 2011) but also in Drosophila, where telomeric retroelements are stimulated in proliferative diploid cells (George & Pardue 2003; Walter & Biessmann 2004). Telomerase activity was tested in two arthropod species - in a lobster (*Homarus americanus*) (Klapper et al. 1998) and in a cockroach *Periplaneta americana* (Sasaki & Fujiwara 2000), but, surprisingly, no regulation of telomerase activity in a tissue-specific manner has been observed.

The aim of the thesis was, therefore, mapping telomerase activity across insects to confirm telomerase as the major telomere maintenance mechanism in insect and quantifying telomerase activity in different developmental stages and different tissues of a cockroach *P. americana* and a honeybee *Apis mellifera* as representatives of hemi- and holometabolous insects, respectively.

2) Telomerase activity regarding caste specific differences in longevity in *A. mellifera*

Several endocrine pathways have been suggested to be involved in lifespan regulation and caste differentiation in honey bees. Surprisingly, no information has been known about a role

of telomerase in honey bees although it is known that telomerase is one of key regulators of lifespan in vertebrates.

The aim of the thesis was to compare telomerase activity between honey bee queens and honey bee workers as the long-lived caste and short-lived caste, respectively, to clarify if telomerase underlies in the extended lifespan of honey bee queen.

3) Chronic low-dose pro-oxidant treatment stimulates transcriptional activity of telomeric retroelements and increases telomere length in *D. melanogaster*

There might be two aspects regarding the effect of free radicals on telomeres. First, telomeres are highly sensitive to oxidative DNA damage, and therefore oxidative stress can substantially contribute to telomere shortening (reviewed in Houben et al. 2007). Second, if stress agents affect an organism over a long period but in low dose, these agents may stimulate a beneficial "eustress" response on cellular or physiological functions, a phenomenon, which is known as hormesis (Calabrese 2004; Mattson 2008).Whether telomere length and activity of telomere compensation mechanisms are under hormetic effect of low doses of free radicals has not been discovered yet.

The aim of the thesis was to test whether chronic low-dose pro-oxidant treatment stimulates transcriptional activity of telomeric retroelements and might increase telomere length in *D. melanogaster*.

3. MATERIAL AND METHODS

3.1. Specimens

Apis mellifera. *Apis mellifera mellifera carnica* (Hymenoptera: Apidae) was used. Samples were provided by Bee Research Institute at Dol (Czech Republic) and two private beekeepers from Písek (South Bohemia), or samples were collected in the apiary located at Biology Centre AS CR, České Budějovice. The collected samples were frozen in liquid nitrogen and stored in -80 ^{0}C.

Drosophila strains. We used the isogenic wild type Oregon R line maintained at 25°C on cornmeal-molasses medium with dry yeast added to the surface, 60–75% relative humidity, and a 12 hr light/dark cycle. The y^1w^{67c23}*; HeTom* line was prepared by insertion of the *HeTom* construct into position 86E of the y^1 w^{67c23} line.

Other insect specimens. Species and sources are listed in Table 1.

Tab. 1: Tested species and they origin.

Taxon	Species	Origin
Hymenoptera	*Apis mellifera*	Collected by authors near Plzeň, West Bohemia, Czech Republic
	Formica rufa	Collected by authors near Plzeň, West Bohemia, Czech Republic
Lepidoptera	*Ephestia kuehniella*	From cultures at the Institute of Entomology
Trichoptera	*Limnephilus decipiens*	Collected by authors near České Budějovice, South Bohemia, Czech Republic
Coleoptera	*Leptinotarsa decemlineata*	Collected by authors near Plzeň, West Bohemia, Czech Republic
	Graphodeus cinereus	Collected by Dr. Bezděk near České Budějovice, South Bohemia, Czech Republic
	Ips typographus	Collected by Dr. Doležal in Šumava Mts., South Bohemia, Czech Republic
	Silpha obscura	Collected by authors near Plzeň, West Bohemia, Czech Republic
Sternorrhyncha	*Aphid fabae*	Provided by Dr. Havelka from cultures at the Institute of Entomology
Orthoptera	*Locusta migratoria*	Pet shop
	Acheta domestica	Pet shop
	Gryllus assimilis	Pet shop
Isoptera	*Prorhinotermes simplex*	From cultures at the Institute of Organic Chemistry and Biochemistry ASCR, Prague, provided by Dr. Šobotník
Phasmida	*Carausius morosus*	Pet shop
Blattodea	*Periplaneta americana*	From cultures at the Institute of Entomology
Zygentoma	*Lepisma saccharina*	Collected by authors in household

3.2. Preparation of protein extracts

For preparation of protein extracts, we used protocol published by Sasaki and Fujiwara (2000). Briefly, adult head, brains, fat body, ovaries or embryos were homogenized in 200 µl of extraction buffer (10 mM Tris/HCl, pH 7.6; 1 mM MgCl2; 1 mM EGTA; 0.1 mM PMSF; 5 mM β-mercaptoethanol; 0.5% (w/v) CHAPS; 10% (v/v) glycerol and 40 U/ml RNase inhibitor (Promega)). The homogenates were incubated on ice for 30 min and then centrifuged (12,000 g; 20 min; 4 °C). The supernatant was collected and frozen in liquid nitrogen. Aliquots were stored at -80 °C. The concentration of protein was determined by Protein assay kit (Bio-Rad). We tested bee head, queen brains, fat body or ovaries, or 20 embryos as one sample for TRAP. All experiments contain at least five independent samples and were repeated three times.

3.3. TRAP assays (Telomeric Repeat Amplification Protocol)

Briefly, a forward TS primer (sequences 5´-AAGCCATCGAGCAGAGTT- 3´) was used as a substrate for telomerase. The elongation product was amplified by PCR with TS primer and Bm-CXa primer (5'-GTGTAACCTAACCTAACC-3'). We used a Light Cycler CFX96 BioRad Real-time PCR system for detection of TRAP products. Each reaction was performed in 25 µl and contained 5 µg of protein, 0.5 µl of 10 pmol TS primer and 12.5 µl Xceed qPCR 2x Mix (IAB, Institute of Applied Biotechnologies). Samples were incubated at 30 °C for 60 min. For PCR, 0.5 µl of 10 pmol Bm-CXa primer was added and reactions were performed with 30 cycles of 94 °C for 30 s and 60 °C for 30 s. Each tested sample was prepared from different individual heads or legs from one adult, and analyzed in triplicates. At least two independent experiments were performed with each sample. We added 0.5 µl of 1 µg/µl RNase A (Sigma-Aldrich) to samples to prepare negative controls for telomerase activity. We also included ITAS (internal telomere assay standard) to assay an effect of assay inhibitors in samples. The 100-kb long ITAS (Generi Biotech, Czech Republic) contained at its ends sequences for TS and Bm-CXa primers. Per reaction we used 10-18 mol of ITAS. Products of the PCR reaction were resolved on 12% non-denaturing polyacrylamide gels, stained with ethidium bromide and visualized under UV light. Calculation of the relative telomerase

activity was made according to 2Ct (ITAS) / 2Ct (Sample). Threshold cycle values (Ct) were obtained by quantitative real-time PCR.

3.4. Cloning and sequencing of TRAP products

The products of the TRAP assay were purified with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel) and cloned into pGEM T-easy vector (Promega). Plasmid DNA was isolated using the Nucleospin Plasmid Quick Pure Kit (Macherey-Nagel). The inserts were sequenced using ABI PRISM 3.1 (Applied Biosystems). The primers used were T7 and SP6.

3.5. Preparation of y^1w^{67c23}; HeTom reporter line

The *HeTom* construct bears a reporter gene for the Tomato fluorescent protein (Clontech) under the control of a *HeT-A* promoter. The part with *HeT-A* promoter region was prepared using PCR with primers designed to a published sequence (Danilevskaya et al. 1994) (GenBank accession number U06920). Sequences used for the PCR are 70 to 961 nt. The PCR product was cloned into the pGEM vector (Promega) and sequenced (GenBank accession number KJ081250). The construct is provided with gypsy insulators at its ends to protect the construct against position effect upon its insertion into genome. The *HeTom* construct was cloned into pUAST vector (Brand & Perrimon 1993) and injected to y^1w^{67c23} flies by Rainbow transgenic, Inc. The position of the transgenic construct was mapped, using in situ hybridization, to 86E in the Drosophila genome.

3.6. Paraquat and hydrogen peroxide exposure

We used paraquat, which is a strong redox agent that stimulates production of superoxide anions (Choi et al. 2006; Mollace et al. 2003), in the long-term and acute toxicity assay. In the long-term assay we treated Oregon R flies with the wide range of non-/sub-lethal paraquat concentrations throughout the entire development over five successive generations. Adult flies were collected within two hours after eclosion, males and females were separated, and after three days they were transferred into vials containing 1.35 g Formula 4-24 Instant Drosophila

Medium (Carolina) soaked with 6 ml water containing paraquat (1,1-dimethyl-4,4-bipyridinium dichloride hydrate; Sigma-Aldrich) of concentrations from 10^{-1} mM to 10^{-7} mM paraquat or 6 ml of water as the control. A total of 20 flies (10 males and 10 females) were placed in each vial. For each paraquat concentration ten vials were prepared in parallel. In the acute toxicity assay we determined the mortality rate of adult flies after short-term exposure to high dose of paraquat. Three days after eclosion, flies were transferred into vials containing 2.5 cm Whatman Paper soaked with 400 µl of 1% (w/v) sucrose containing 20 mM paraquat or 400 µl of 1% (w/v) sucrose as the control. Numbers of dead flies were recorded after 24 hours.

When we treated flies with hydrogen peroxide we used 10^{-3}, 10^{-4}, and 10^{-6} % hydrogen peroxide (Sigma-Aldrich) applied to one generation of Oregon R. Otherwise, the assay was proceed in the same way as described for the long-term paraquat treatment.

3.7. Extraction of nucleic acid and cDNA synthesis

Genomic DNA was extracted using the E.Z.N.A. Insect DNA kit (Omega bio-tek) and total RNA samples were prepared using a Nucleospin RNA II kit (Macherey-Nagel). For each RNA sample we used twenty adult males of *D. melanogaster* or head, brain, fat body and ovaries of *A. mellifera*. *cDNA* synthesis was performed using 1 *µg total RNA* primed with oligo(dT) and Superscript II reverse transcriptase (Invitrogen).

3.8. Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed to evaluate relative transcript levels and genomic copy numbers of Drosophila telomeric elements as described previously (Capkova Frydrychova et al. 2007; Pfaffl 2001) or to measure telomerase activity and evaluate relative transcript level of honey bee hormones. For all experiments, we used a Light Cycler CFX96 BioRad real-time PCR system and each reaction was performed in 25 µl and contained 1 µg cDNA, 0.5 µl of 10 pmol reverse primer and 0.5 µl of 10 pmol forward primer and 12.5 µl (Top-Bio) SYBR Premix Ex Taq™ II (Takara) or 2x SYBR Master Mix (Top-Bio). At least two independent experiments analyzed in triplicates were performed with each sample. We calculated the level

of expression from C_T values of target sequences and reference genes and with correction for amplification efficiency (Pfaffl 2001) according to $2^{Ct\ (reference)} / 2^{Ct\ (sample)}$.

As the genes of reference for honey bee were used already published RpL13α and actin (Scharlaken et al. 2008; Lourenço et al. 2008). The results from both pairs of primers correspond to each other. The primers for analysis were designed according to the published sequence of *A. mellifera* and they are recorded in Apendix (see Ap. I.I.). Reactions were performed with 30 cycles of 95 °C for 15 s and 55 °C for 30 s. Each tested sample was prepared from different individual head, brain, fat body or ovaries.

For *D. melanogaster*, the threshold cycle values (Ct) were normalized against *RpL32* or *Actin 42A*, and ΔΔ Ct method with correction for amplification efficiency was used to calculate levels of targets. When we measured transcript levels in our pilot experiments, data were normalized to both internal controls (*RpL32* and *Actin 42A*). As no differences were found between the data resulting from both normalization, in further experiments the normalization was performed using *RpL32* only. To get absolute numbers of elements in the tested genomes, relative numbers of retroelements were normalized against the *white* gene. Sequences of primers are shown in Apendix (see Ap. I.II.). Reactions were performed with 30 cycles of 95 °C for 15 s and 58 °C for 30 s.

3.9. Quantification of fluorescence signals

Transgenic individuals were scored for Tomato fluorescence under stereo-microscope with an appropriate filter. The fluorescent signals were recorded with an Olympus camera SZX12, and intensity of fluorescence was evaluated using Adobe Photoshop 11.0.2.

3.10. Determination of AKH level

The AKH level was determined using the competitive ELISA in extract prepared from dissected *corpora cardiaca* attached to brain and *corpora allata* as described previously (Zemanová et al. 2016).

3.11. Viability assay (egg to adult survival)

The viability assay was performed after the long-term paraquat treatment by scoring newly eclosed flies of the fifth generation. It was done in two ways. First, we counted adults eclosed from eggs that had been laid by ten females directly in the tested vials. Second, we counted adults eclosed from eighty eggs that we had transferred into the tested vials.

3.12. Statistical analysis

Statistical analyzes were performed using Unpaired t-test, One-way ANOVA or Two-way ANOVA with Bonferroni post-test using GraphPad Prism 4 (GraphPad Software., San Diego, CA). The types of using statistical test in individual analyzes are described in legends under each Figures. The values in the graphs represent the mean of measurement ± SE. All analyzes were done at least from two independent attempts.

4. RESULTS

4.1. Published results

4.1.1. Publication no. 1

Korandová M, Krůček T, Vrbová K, Čapková Frydrychová R (2014). Distribution of TTAGG-specific telomerase activity in insects. Chromosome Research 22: 495-503.

Abstract. In most eukaryotes, telomeres consist of tandem arrays of a short repetitive DNA sequence. Insect telomeres are generally constituted by a (TTAGG)n repeat motif. Usually, telomeres are maintained by telomerase, a specialized reverse transcriptase that aids this sequence to chromosome ends. We examined telomerase activity in 15 species across Insecta. Telomerase activity was revealed in Isoptera, Blattaria, Lepidoptera, Hymenoptera, Trichoptera, Coleoptera, and Sternorrhyncha. In contrast, we were not able to detect telomerase activity in Orthoptera, Zygentoma, and Phasmida. Because we found telomerase activity in phylogenetically distant species, we conclude that a distribution pattern of (TTAGG)n sequence in Insecta is generally consistent with that of telomerase activity. Thus, the TTAGG-telomerase system is functional across the Insecta. Using real-time quantitative telomeric repeat amplification protocol (RTQ-TRAP) system, we quantified telomerase activity in different developmental stages and different tissues of a cockroach, *Periplaneta a**mericana*. We show that telomerase is upregulated in young instars and gradually declines during development. In adults, it is most active in testes and ovaries. Thus, the telomerase activity of hemimetabolous insects seems to be associated with cell proliferation and organismal development.

4.1.2. Publication no. 2

Korandová M, Čapková Frydrychová R (2016). Telomerase activity and telomere length in *Apis mellifera*. Chromosoma 125: 405-411.

Abstract. Telomerase is an enzyme that adds repeats of DNA sequences to the ends of chromosomes, thereby preventing their shortening. Telomerase activity is associated with proliferative status of cells, organismal development, and aging. We report an analysis of telomerase activity and telomere length in the honeybee, *Apis mellifera*. Telomerase activity was found to be regulated in a development and caste-specific manner. During the development of somatic tissues of larval drones and workers, telomerase activity declined to 10 % of its level in embryos and remained low during pupal and adult stages but was upregulated in testes of late pupae, where it reached 70 % of the embryo level. Upregulation of telomerase activity was observed in the ovaries of late pupal queens, reaching 160 % of the level in embryos. Compared to workers and drones, queens displayed higher levels of telomerase activity. In the third larval instar of queens, telomerase activity reached the embryo level, and an enormous increase was observed in adult brains of queens, showing a 70-fold increase compared to a brain of an adult worker. Southern hybridization of terminal TTAGG fragments revealed a high variability of telomeric length between different individuals, although the same pattern of hybridization signals was observed in different tissues of each individual.

4.2. Unpublished data

4.2.1. Telomerase activity in correlation with caste specific lifespan differences in honey bee *Apis mellifera*

Korandová M, Valchářová J, Krištůfek V, and Čapková Frydrychová R

Abstract. Although based on mostly vertebrate research, telomerase is generally thought to be an important lifespan factor. In *Apis mellifera* we asked whether telomerase activity might underlie the caste specific lifespan differences and is changed during honey bee aging. Using TRAP we tested telomerase activity in different-aged workers collected during summer, and in short- and long-lived summer and winter workers, respectively. The data showed no differences in telomerase activity during honey bee aging, however, telomerase activity changed during a year with up-regulation during cold season. The insect fat body is a dynamic tissue involved in multiple metabolic functions, playing crucial roles in the insect life. When evaluating telomerase activity in the abdominal fat body of queens we found telomerase up-regulation compared to workers. The fat body of queens showed a decline in transcript levels of InR, TOR and juvenile hormone, which are pro-aging factors in honey bees, and by contrast, levels of vitellogenin, which has anti-aging effect, was increased.

Results and discussion

Samples of *A. mellifera* workers were collected each month from November 2014 to April 2016, and telomerase activity was evaluated using TRAP assay in heads of the collected bees. The results showed that telomerase activity is up-regulated in bees during cold season, where more than 2-fold increase of telomerase activity was observed. The statistical significant increase of telomerase activity was found in November – December 2014 and 2015 and January – February 2016 (compared to the period of May – October 2015) (Fig. 1). Together with telomerase, transcript levels of juvenile hormone, TOR (Target of Rapamycin), InR (Insulin-like Receptor) and vitellogenin were measured, however, transcript levels did not reveal any differences between tested samples (data not shown). Also, no differences were

detected when we measured telomerase activity in different-aged workers collected from May to September 2017 (data not shown).

Next, we analyzed telomerase activity and transcript levels of several endocrine signalling factors in the fat body of queens and workers. The fat body is an organ of substantial biosynthetic and metabolic activity. Besides synthesis and utilization of energy reserves, the insect fat body produces most of the hemolymph proteins and circulating metabolites**,** and in both vertebrates and invertebrates the fat body is connected to regulation of lifespan (reviewed in Arrese & Soulages 2010). In the fat body of queens we found a roughly two-fold increase ($P = 0.0006$) of telomerase activity (Fig. 2), and consistently with published data (Corona et al. 2007) we also observed a substantial increase of vitellogenin ($P = 0.0253$) (Fig. 3A) and a decrease of juvenile hormone ($P = 0.0138$) (Fig. 3B) and InR ($P = 0.0002$) (Fig. 3D). Reduced transcript levels in the fat body of queens were also observed when we analyzed TOR (a 2.5 - fold decrease, $P = 0.0358$) (Fig. 3C).

Research in variety of organisms suggests interplay between reproduction and lifespan through fat metabolism, and the loss of reproductive system significantly increases both lipid storage and lifespan in vertebrate and invertebrate animals (reviewed in Arrese & Soulages 2010). Negative effects of insulin/IGF-1 signalling and the TOR pathway on both lifespan and fat storage have been observed in various organisms ranging from budding yeast to mammal, suggesting an evolutionarily conserved axis (Hansen et al. 2013; Wei et al. 2013). Oxidative stress is considered as a major contributor to aging. Recently, several studies reported an association between the TOR and IIS inhibition and oxidative stress response. First, Budanov & Karin (2008) demonstrated a link between oxidative stress, p53 and mTOR signalling pathway. Tumor suppressor p53 is activated upon oxidative stress and in turn inhibits cell proliferation and growth, and activates sestrins, which through the interaction with TSC1:TSC2 complex (tuberous sclerosis complex) and AMP-responsive protein kinase lead to the TOR inhibition. Second, the TOR inhibition elevates transcript levels of oxidative stress response genes, such as *superoxide dismutase 1* (*sod1*) (Kofman et al. 2012). As found by Zarse et al. (2012) glucose restriction and the impairment of IIS in *Caenorhabditis elegan*s had the life- and health-promoting effect through mitohormetic effect by the elevation of reactive oxygen species generated by mitochondria. It was hypothesised that depletion of

intracellular glucose is sensed by AMP-activated protein kinase, which induces oxidative non-glucose metabolism leading to the production of reactive oxygen species (Zarse et al. 2012).

In honey bees, the fat body produces vitellogenin, which is an antioxidant and important determinant of the extended lifespan of honey bee queens. Numerous studies showed that level of vitellogenin strongly drops during nurse/forage transition, whereas it is enhanced in long-lived winter bees and queens (Amdam & Omholt 2003; Amdam et al. 2004; Amdam et al. 2005). However, our study did not reveal any increase in vitellogenin on its transcript levels either in winter bees or nurses, and, thus, we can hypothesise that vitellogenin level does not reflect its transcript level. However, we saw a significant increase of vitellogenin transcript levels in queens, which presumably demonstrates that a high abundance of vitellogenin in honey bee queens is associated with a strong boosting of its mRNA level.

Honey bee queens are fed with royal jelly throughout their whole life, while adult workers feed honey and bee pollen (Page & Peng 2001). Royal jelly is a bioactive substance composed of 60–70% water, 12–15% crude protein (nine most abundant proteins are termed as MRJ proteins, major RJ proteins), 10–16% sugar, 3–6% lipids and traces of vitamins, salts and free amino acids (reviewed in Pasupuleti et al. 2017). The exclusive feeding of honey bee larvae with royal jelly is the crucial determinant of queen development and longevity of honey bee queens, and providing low doses of royal jelly to honey bee workers results in their extended life. Longevity extension effects of royal jelly was even observed in mice, *D. melanogaster* and *C. elegans* (Inoue et al. 2003; Chen et al. 2009; Kamakura 2011; Honda et al. 2011), and numerous reports showed an effect of royal jelly on cell proliferation, stem cells differentiation, and tissue regeneration (Hattori et al. 2007; Salazar-Olivo & Paz-González 2005). Although the insect fat body is considered to be composed mainly of polyploid cells, we can speculate that royal jelly may induce cell proliferation or differentiation of stem cells, possibly presented in the fat body of queens, to ensure a tissue renewal on a long term basis, and thus explaining high levels of telomerase in the queen fat body we observed.

However, our unpublished data showed the elevated levels of telomerase activity also in the fat body of kings and queens of termite *Prorhinotermes simplex* and queens of bumble bee *Bumbus terrestris*. This may indicate that the up-regulation of telomerase activity in the fat body seems to be a common phenomenon for the reproductive individuals of social insect. The causal link between telomerase activity and the fat body of honey bee queens, as well as

the up-regulation of telomerase during cold season need to be elucidated in a more detail study. Nevertheless, based on these data we can postulate that a caste-specific manner exists in regulation of telomerase in social insects.

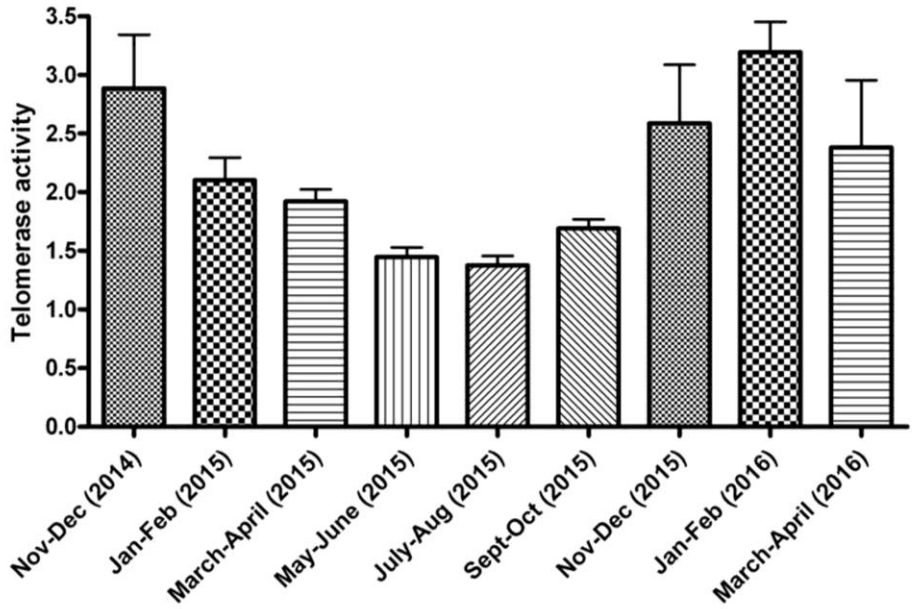

Figure 1: Activity of telomerase in *A. mellifera* heads. Telomerase activity was measure in five hives every month during one and half year (2014/2016). The data from periods Nov-Dec 2014, Nov-Dec 2015, and Jan-Feb 2016 are statistically different from the May-Oct 2015 ($P < 0.05$). The overall results of Bonferonni´s multiple comparison test are attached in Apendix (See Ap. II)

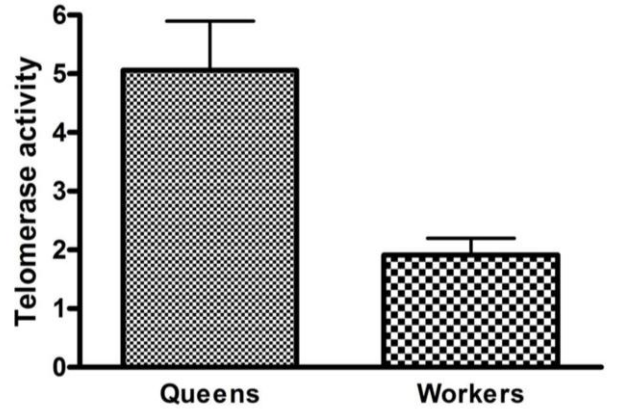

Figure 2: Comparison of telomerase activity between the queen and worker fat bodies. The activity of telomerase in the fat body of queen and workers was measured using TRAP assay. Data was evaluated by Unpaired t-test ($P = 0.0006$; $Df = 13$).

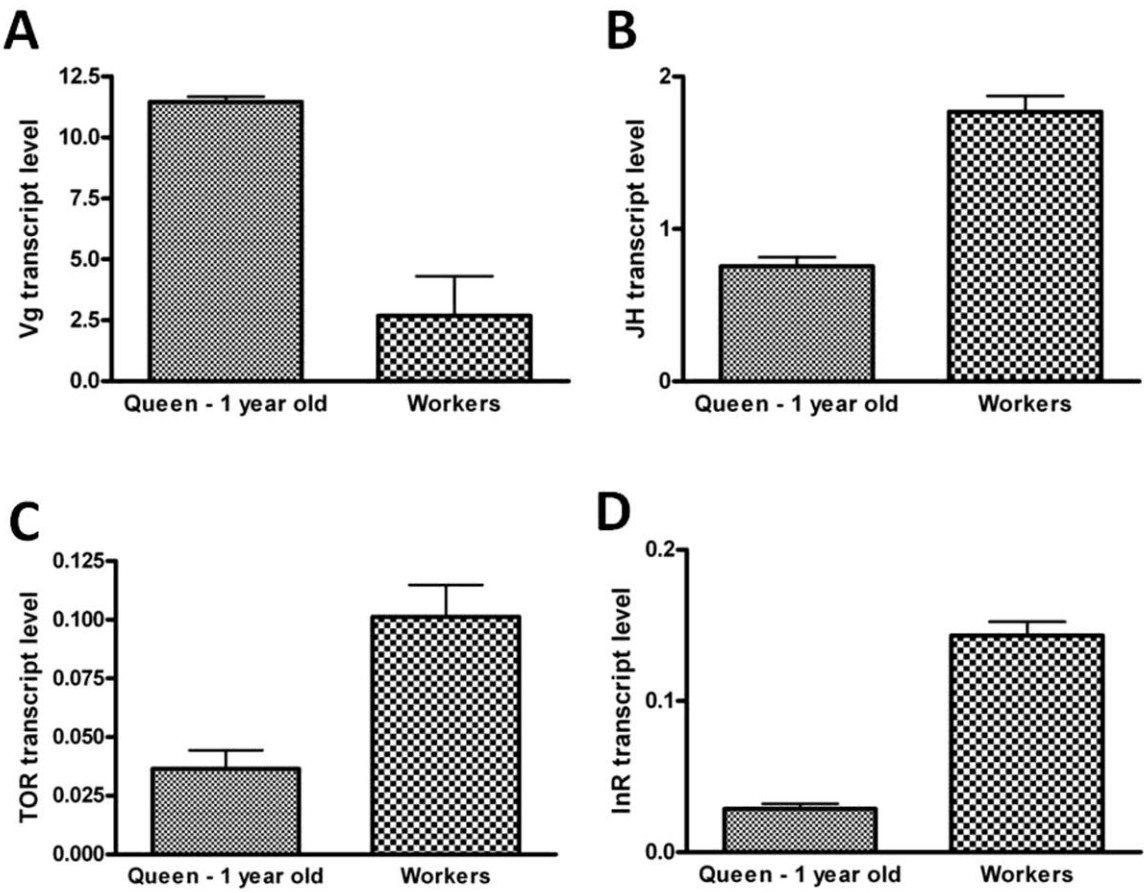

Figure 3: The transcript levels in the fat body of queens and worker bees. We measured transcript levels of vitellogenin (A), juvenile hormone (B), TOR kinase (C) and InR (D). Statistical analyses was performed using unpaired t-tests; vitellogenin ($P = 0.0253$; $Df = 5$), juvenile hormone ($P = 0.0138$; $Df = 5$), TOR ($P = 0.0358$; $Df = 5$) and InR (D) ($P = 0.0002$; $Df = 5$).

4.2.2. Chronic low-dose pro-oxidant treatment stimulates transcriptional activity of telomeric retroelements and increases telomere length in Drosophila

Authors: **Korandová M.**, Krůček T., Szakosová K., Kodrík D., Kühnlein R. P., Tomášková J., Čapková Frydrychová R (2017, manuscript).

Abstract. It has been proposed that oxidative stress, elicited by high levels of reactive oxygen species, accelerates telomere shortening by erosion of telomeric DNA repeats. While most eukaryotes counteract telomere shortening by telomerase-driven addition of these repeats, telomeric loss in Drosophila is compensated by retrotransposition of the telomeric retroelements *HeT-A*, *TART* and *TAHRE* to chromosome ends. In this study we tested the effect of chronic exposure of flies to non-/sub-lethal doses of paraquat, which is a redox cycling compound widely used to induce oxidative stress in various experimental paradigms including telomere length analyses. Indeed, chronic paraquat exposure for five generations resulted in elevated transcriptional activity of both telomeric and non-telomeric transposable elements, and extended telomeric length in the tested fly lines. We propose that low oxidative stress leads to increased telomere length within Drosophila populations. For a mechanistic understanding of the observed phenomenon we discuss two scenarios: adaption, acting through a direct stimulation of telomere extension, or positive selection favouring individuals with longer telomeres within the population.

The results have been submitted in a form manuscript to Journal of Insect Physiology.

Chronic low-dose pro-oxidant treatment stimulates transcriptional activity of telomeric retroelements and increases telomere length in Drosophila

Michala Korandová*§, Tomáš Krůček*§, Klára Szakosová*, Dalibor Kodrík*§, Ronald P. Kühnlein**[2,3], Jindřiška Tomášková* and Radmila Čapková Frydrychová*§[1]

Address:

* Institute of Entomology, Biology Centre AS CR, České Budějovice, 37005, Czech Republic, § Faculty of Science, University of South Bohemia, České Budějovice, 37005, Czech Republic

**Max-Planck-Institut für biophysikalische Chemie, Research Group Molecular Physiology, D-37077 Göttingen, Germany

[2] Current address: Institute of Molecular Biosciences, University of Graz, A-8010 Graz, Austria

[3] BioTechMed-Graz, Graz, Austria

Running title: Oxidizing agents stimulate telomere lengthening

Key words: Drosophila, oxidative stress, telomeres, hydrogen peroxide, paraquat, hormesis

[1] Corresponding author: Radmila Čapková Frydrychová, Institute of Entomology, Biology Centre AS CR, Branišovská 31, České Budějovice, 37005, Czech Republic, phone number: +420 387 775 054, fax number: +420 385 310 354, Radmila.Frydrychova@hotmail.com.

Abstract

It has been proposed that oxidative stress, elicited by high levels of reactive oxygen species, accelerates telomere shortening by erosion of telomeric DNA repeats. While most eukaryotes counteract telomere shortening by telomerase-driven addition of these repeats, telomeric loss in Drosophila is compensated by retrotransposition of the telomeric retroelements *HeT-A*, *TART* and *TAHRE* to chromosome ends. In this study we tested the effect of chronic exposure of flies to non-/sub-lethal doses of paraquat, which is a redox cycling compound widely used to induce oxidative stress in various experimental paradigms including telomere length analyses. Indeed, chronic paraquat exposure for five generations resulted in elevated transcriptional activity of both telomeric and non-telomeric transposable elements, and extended telomeric length in the tested fly lines. We propose that low oxidative stress leads to increased telomere length within Drosophila populations. For a mechanistic understanding of the observed phenomenon we discuss two scenarios: adaption, acting through a direct stimulation of telomere extension, or positive selection favoring individuals with longer telomeres within the population.

1. Introduction

Normal cellular processes, such as incomplete DNA replication or oxidative damage, may lead to gradual loss of chromosome termini. Telomeres, special ribonucleoprotein structures at chromosome ends, are essential to solve the chromosome end shortening problem and thus play a key role in chromosome stability (Denchi, 2009; Chan and Blackburn, 2002; von Zglinicki, 2002). The most common mechanism of compensation for telomere shortening is

activity of telomerase, the enzyme that adds short repetitive DNA sequences to chromosome ends (Nandakumar & Cech 2013; Mason et al. 2016). Telomerase activity is related to the cell proliferation status and it is mostly found in highly dividing cells such as embryonic cells, germ cells or stem cells. Disruption of telomerase activity is connected to aging processes and cancer (Wright et al., 1996; Zhu et al., 2011).

A different telomere maintenance mechanism has been identified in *Drosophila melanogaster* that lacks telomerase. Drosophila telomeres are composed of arrays of telomeric retrotransposons that compensate for telomeric loss by their retrotransposition to chromosome ends. There are three telomere-specific retroelements found in *D. melanogaster*, *HeT-A*, *TAHRE* and *TART* (collectively abbreviated HTT) that are present in multiple copies on each chromosome end (Capkova Frydrychova et al., 2009; Mason et al., 2008). Transcription of telomeric retroelements, which is a key step in telomere elongation in Drosophila, has been found to correlate with cell proliferation (Danilevskaya et al., 1997; Pardue and DeBaryshe, 2003; Walter and Biessmann, 2004).

Telomere length is maintained through the interplay between telomere extension mechanisms and telomere shortening events. It has been proposed that human telomere length is modulated by various endogenous and exogenous factors, such as emotional or physical stress, health, diet, aging, etc. (Zhu et al., 2011). Oxidative stress is regarded as the main cause of telomere shortening; its impact on telomere length has been proposed to be even larger than telomere loss during DNA replication (von Zglinicki, 2002). Endogenous oxidative stress is associated with several cellular processes, such as the oxidative phosphorylation in mitochondria or inflammation, and its level is modulated by various endogenous and exogenous factors (Cui et al., 2012). *In vitro* mitochondrial dysfunction-induced reactive oxygen species (ROS) or hyperoxia lead to accelerated telomere shortening

and reduce proliferative lifespan of cultured somatic cells (Passos et al., 2007). The level of oxidative stress is related to the cellular capacity for anti-oxidative defense (Missirlis et al., 2001).

Almost all information about a stress impact on telomeres comes from research on humans. However, it seems that the influence of stress on telomeric activity is not limited to humans and acts regardless the type of telomeric DNA sequence or telomere compensation mechanism. We can predict a stress response on telomeric level also for invertebrates. In Chironomus, where telomeres consist of long satellite sequences and are maintained by homologous recombination, telomeric transcription activity has been found to be increased upon environmental stress (Martínez-Guitarte et al., 2008).

In our study we tested the effect of paraquat, the pro-oxidant leading to superoxide anion production, applied in low doses to five successive generations of flies. Besides the increased resistance of flies to lethal paraquat doses, we found that long term paraquat treatment increased transcriptional activity of HTT elements and non-telomeric elements resulting in extended telomeric length. Similarly, the elevation of telomeric transcript levels was observed after exposure to low doses of H_2O_2. Therefore, we propose that pro-oxidants might act in a hormetic fashion on telomere length, with telomere erosion in response to high doses and telomere elongation in response to low doses, respectively.

2. Materials and methods

2.1. Drosophila strains.

We used the isogenic wild type Oregon R line maintained at 25°C on cornmeal-molasses medium with dry yeast added to the surface, 60–75% relative humidity, and a 12 hr

light/dark cycle. The y^1w^{67c23}; *HeTom* line was prepared by insertion of the *HeTom* construct into position 86E of the $y^1\ w^{67c23}$ line.

2.2. Preparation of y^1w^{67c23}; HeTom reporter line

The *HeTom* construct bears a reporter gene for the Tomato fluorescent protein (Clontech) under the control of a *HeT-A* promoter. The part with *HeT-A* promoter region was prepared using PCR with primers designed to a published sequence (Danilevskaya et al., 1994) (GenBank accession number U06920). Sequences used for the PCR are nt 70 to 961. The PCR product was cloned into the pGEM vector (Promega) and sequenced (GenBank accession number KJ081250). The construct is provided with gypsy insulators at its ends to protect the construct against position effect upon its insertion into genome. The *HeTom* construct was cloned into pUAST vector (Brand and Perrimon, 1993) and injected to y^1w^{67c23} flies by Rainbow transgenic, Inc. The position of the transgenic construct was mapped, using in situ hybridization, to 86E in the Drosophila genome.

2.3. Paraquat and hydrogen peroxide exposure

We used paraquat, which is a strong redox agent that stimulates production of superoxide anions (Choi et al., 2006; Mollace et al., 2003), in the long-term and acute toxicity assay. In the long-term assay we treated Oregon R flies with the wide range of non-/sub-lethal paraquat concentrations throughout the entire development over five successive generations. Adult flies were collected within two hours after eclosion, males and females were separated, and after three days they were transferred into vials containing 1.35 g Formula 4-24 Instant Drosophila Medium (Carolina) soaked with 6 ml water containing paraquat (1,1-dimethyl-4,4-bipyridinium dichloride hydrate; Sigma-Aldrich) of concentrations from 10^{-1} mM to 10^{-7}

mM paraquat or 6 ml of water as the control. A total of 20 flies (10 males and 10 females) were placed in each vial. For each paraquat concentration ten vials were prepared in parallel. In the acute toxicity assay we determined the mortality rate of adult flies after short-term exposure to high dose of paraquat. Three days after eclosion, flies were transferred into vials containing 2.5 cm Whatman Paper soaked with 400 µl of 1% (w/v) sucrose containing 20 mM paraquat or 400 µl of 1% (w/v) sucrose as the control. Numbers of dead flies were recorded after 24 hours.

When we treated flies with hydrogen peroxide we used 10^{-3}, 10^{-4}, and 10^{-6} % hydrogen peroxide (Sigma-Aldrich) applied to one generation of Oregon R. Otherwise, the assay was proceed in the same way as described for the long-term paraquat treatment.

2.4. Extraction of nucleic acid and cDNA synthesis

Genomic DNA was extracted using the E.Z.N.A. Insect DNA kit (Omega bio-tek) and total RNA samples were prepared using a Nucleospin RNA II kit (Macherey-Nagel). For each RNA sample we used twenty adult males. cDNA synthesis was performed using 1 µg total RNA primed with oligo(dT) and Superscript II reverse transcriptase (Invitrogen).

2.5. Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed to evaluate relative transcript levels and genomic copy numbers of telomeric elements as described previously (Capkova Frydrychova et al., 2007; Pfaffl, 2001). We used a Light Cycler CFX96 BioRad real-time PCR system and SYBR Premix Ex Taq^{TM} II (Takara). Threshold cycle values (Ct) were normalized against *RpL32* or *Actin 42A*, and ΔΔ Ct method with correction for amplification efficiency was used to calculate levels of targets. When we measured transcript levels in our pilot experiments, data

were normalized to both internal controls (*RpL32* and *Actin 42A*). As no differences were found between the data resulting from both normalization, in further experiments the normalization was performed using *RpL32* only. To get absolute numbers of elements in the tested genomes, relative numbers of retroelements were normalized against the *white* gene. Sequences of primers are shown in Table S1.

2.6. Quantification of fluorescence signals

Transgenic individuals were scored for Tomato fluorescence under stereo-microscope with an appropriate filter. The fluorescent signals were recorded with an Olympus camera SZX12, and intensity of fluorescence was evaluated using Adobe Photoshop 11.0.2.

2.8. Determination of AKH level

The AKH level was determined using the competitive ELISA in extract prepared from dissected *corpora cardiaca* attached to brain and *corpora allata* as described previously (Zemanová et al., 2016).

2.9. Viability assay (egg to adult survival)

The viability assay was performed after the long-term paraquat treatment by scoring newly eclosed flies of the fifth generation. It was done in two ways. First, we counted adults eclosed from eggs that had been laid by ten females directly in the tested vials. Second, we counted adults eclosed from eighty eggs that we had transferred into the tested vials.

2.10. Statistical analysis.

Statistical analyses were done using GraphPad Prism 4.0 (GraphPad Software Inc.) by One-way ANOVA followed by Dunnett's Multiple Comparison Test or Two-way ANOVA followed by Bonferroni post-hoc test; the type of test used in individual analysis is specified in the figure legends.

3. Results

3.1. Multi-generational exposure to non-/sub-lethal paraquat doses increases telomere length and transcriptional activity of telomeric elements

By analyzing the genomic copy number of telomeric elements as marker of telomere length, we found that the telomere length was increased after multi-generational exposure to non-/sub-lethal paraquat concentrations. Statistical significant elevations of *HeT-A* copy numbers started being observed in the 3rd generation of flies that had been exposed to 10^{-3} and 10^{-4} mM paraquat, and copy numbers showed an increasing tendency during the 4th and 5th generations we further followed (Fig. 1A, S2-6). While in the 3rd generation 10^{-4} mM paraquat treated flies revealed *HeT-A* copy number increase by 30 %, in the 5th generation increase was by 70 % ($P < 0.01$). Also, 10^{-4} mM paraquat treated flies displayed an increase of *TART* and *TAHRE* copy numbers. In the 5th generation we found an increase by 60 % ($P < 0.05$) and 35 % ($P < 0.05$) of *TART* (Fig. 1B, Supplemental data 7-8) and *TAHRE* numbers (Fig. 1C, Supplemental data 9-10), respectively.

Then, we asked if the observed HTT copy number increase might be associated with elevation of HTT transcript levels. We tested transcript levels of *HeT-A* in flies of the 1st and 5th generations. In contrast to the 1st generation, where we did not observe any statistical significant change in transcript levels after paraquat treatment (Fig. 2A, Supplemental data

S11), the 5th generation of flies revealed that treatment with 10^{-3} and 10^{-4} mM paraquat increased the *HeT-A* transcript levels roughly by three-fold compared to the control group ($P < 0.001$) (Fig. 2B, Supplemental data S11). Similarly, the increase of *HeT-A* transcript levels in response to the corresponding paraquat treatment was observed in the genetically unrelated *y^1 w^{67c23}; HeTom* line (Fig. 2C, Supplemental data S12). Then, we asked whether the levels of *HeT-A* transcript relative to the *HeT-A* genomic copy number change during the long term paraquat treatment. Therefore, we calculated a ratio between *HeT-A* transcript level and number of genomic *HeT-A* in the 1st and 5th generation, and we found that the level of *HeT-A* transcript increase per element is in both generations without an overall statistically significant difference (Supplemental data Fig. S1).

3.2. Non-/sub-lethal doses of paraquat also stimulate the transcriptional activity of non-telomeric retroelements

To address potential general activation effect of the chronic non-/sub-lethal paraquat treatment, we evaluated transcript levels from the non-telomeric *HeTom* transgene insertion, which carries the *Tomato* reporter gene under control of *HeT-A* promoter. In accordance with the observed telomeric transcriptional activity of *HeT-A*, increased *HeTom* transcript levels were found in the flies treated with 10^{-3} and 10^{-4} mM paraquat ($P < 0.05$) (Fig. 2D, Supplemental data S13). Using fluorescence signal we followed the intensity and localization of *HeTom* expression during larval development of the tested flies. Slight fluorescence was detected in brain and imaginal discs of the 3rd instar larvae (not shown), however, based on inconsistent differences in the localization and intensity of fluorescence we did not analyze it further. Nevertheless, strong fluorescence was consistently seen in larval testes. Here, when we quantified the average intensity of fluorescence we found a fluorescence signal increase

after the paraquat treatment ($P < 0.0001$) (Fig.3A, B, Supplemental data S14). Comparison of individual larvae showed that the distribution of fluorescence intensity was shifted towards higher levels in flies treated with paraquat compared to the control flies (Fig. 3C-F). To assess autofluorescence, we concurrently examined control y^1w^{67c23} and Oregon R individuals, but no fluorescence was observed.

Also, we tested paraquat-treated Oregon R flies on transcriptional activation of the non-telomeric retroelement *Jockey* (Fig. 4, Supplemental data S15). A statistically significant effect was observed in the 5th generation, where an increase of *Jockey* transcript levels was observed in flies treated with either 10^{-3} mM paraquat (2.5-fold increase, $P < 0.05$) or with 10^{-6} mM paraquat (three-fold increase, $P < 0.01$). However, in spite of the increase of *Jockey* transcript levels we did not observe any significant increase in its genomic copy number (Fig. S2).

3.3. Telomeric transcript activity is also stimulated by low doses of hydrogen peroxide

To confirm our premise that the observed changes in telomere activity might be caused by response to increased levels of reactive oxygen species, we next treated flies with low doses of another oxidizing agent, hydrogen peroxide. Exposure of Oregon R flies to 10^{-3} % hydrogen peroxide for one generation resulted in the increase of *HeT-A* transcript levels by 1.9-fold ($P < 0.01$) compared to the control (Fig. 5, Supplemental data S16). This result confirms that mild long-term oxidative treatment leads to stimulation of telomeric transcriptional activity independent of the chemical nature of the oxidizer.

3.4. Is the observed increase of HTT copy numbers result of an adaption, acting through a direct stimulation of telomere elongation, or rather selection mechanism?

Supported by the *HeTom* transcript elevation, the increased levels of HTT copy numbers and HTT transcripts can be explained as direct stimulation of telomere activity and elongation, which might acts as a part of oxidative stress adaptation. But reversely, we could also explain it as long-term selection that eliminates individuals with shorter telomeres from the population. We assumed that if the effect of long-term selection is the case, changes in numbers of eclosed flies should be seen. Therefore, we scored the 5^{th} generation flies using the viability assay, and the experiment was performed in two ways. First, when we counted flies eclosed from eighty eggs, which we had collected from the tested groups and transferred into fresh vials, we found only slight differences without statistical significance (Fig.6A, Supplemental data S18). However, scoring flies in vials where eggs were directly laid by ten females showed statistical significant decrease in group that had been exposed to 10^{-1} mM paraquat, where the number of flies was around 20 % ($P < 0.01$) of the control. The lower paraquat concentrations tended to produce less progeny than the control, but the differences were not statistically significant (Fig. 6B, Supplemental data S18). Based on the data it seems that chronic paraquat treatment may create selection pressure within population, presumably depending on paraquat concentration and population density.

3.5. Long-term non-/sub-lethal paraquat treatment increases AKH levels as a marker of antioxidant defense

Then, we decided to answer the question if the long-term paraquat treatment is associated with an enhanced antioxidant response. We tested levels of AKH, one of key players in the insect antioxidant defense, which is released upon induction with high oxidant

doses (Bednářová et al., 2013; Večeřa et al., 2007; Velki et al., 2011). First, we analyzed the AKH levels in the 5th generation of flies that had undergone the long-term paraquat assay, and we found no differences in the AKH level between control and paraquat-treated flies (data not shown). However, levels of AKH were elevated when the flies were exposed to a single lethal levels were found in flies chronically exposed to paraquat concentrations from 10^{-4} to 10^{-1} mM, where the highest increase (by 63 %) was recorded for the maximal paraquat concentration (Fig. 7A, Supplemental data S19). When we assessed the mortality after the 20mM paraquat exposure we found that the survival rate in lines that had been exposed to non-/sub-lethal paraquat doses was significantly higher compared to the control group. While the survival rate of control flies reached only to about 40 %, in the paraquat-pretreated lines it was increased up to 75 % (Fig. 7B, Supplemental data S20).

Collectively, chronic non-/sub-lethal paraquat doses enhanced levels of HTT transcript and genomic copy numbers, which could be explained as a result of adaption through direct stimulation of telomere elongation or selection, acting within the tested population, against individuals with short telomeres. Also, long-term treatment with non-/sub-lethal paraquat concentrations is associated with the enhanced protection of flies against lethal doses of paraquat.

4. Discussion

There were two presumptions for this study. First, telomeres are, due to their high guanine content, highly sensitive to oxidative DNA damage, and therefore oxidative stress can substantially contribute to telomere shortening (reviewed in Houben et al., 2007). Second, if harmful chemical, biological or physical agents affect an organism over a long

period but in low dose, these agents may stimulate a beneficial "eustress" effect on cellular or physiological functions, a phenomenon, which is known as hormesis (Calabrese, 2004; Mattson, 2008). A possible hormetic effect of paraquat treatment was suggested in our previous study with Drosophila where a biphasic dose response curve between antioxidant response and paraquat concentrations was observed (Krůček et al., 2015). Despite a lack of published information, we might predict that high levels of reactive oxygen species increase telomere erosion in Drosophila in the same fashion as commonly proposed for mammals (Coluzzi et al., 2014; Oikawa et al., 2001; von Zglinicki, 2002). On the other hand, a possible hormetic effect of oxidizing agents on telomeres is so far completely unclear.

Now, based on the present study we can assert that long-term treatment of Drosophila with optimal concentrations of oxidizing agent not only induces resistance against lethal paraquat doses but it is associated with progressive telomere lengthening and transcriptional activity at telomeres. We can predict a stimulation of telomeric element transposition, as transcriptional activity is one of key steps in this telomere elongation mechanism in Drosophila (Capkova Frydrychova et al., 2009; Mason et al., 2008; Pardue and DeBaryshe, 1999). However, a contribution of gene conversion, the alternative telomere elongation mechanism based on recombination between the homologous telomeric sequences, cannot be ruled out. The long-term paraquat treatment increased telomere length of subsequent generations as well as increased fluorescence level of reporter *HeTom* transgene in testes. This indicates that changes responsible for the observed increase in retrotransposon activity must also occur in the germ line. This assertion is supported by the observation that transcriptional activity of Drosophila telomeric elements is associated with proliferative active cells, such as germ cells (George and Pardue, 2003; Walter and Biessmann, 2004).

Previous studies on various model organisms identified transposable elements as modifiers of the genetic response upon exposure to stressful environment (Mourier et al., 2014). The activity of transposable elements leads to insertional mutagenesis, and if induced by environmental stress, it creates highly efficient adaptive mechanisms to changing conditions and certain stressors. It has long been known that telomeric retroelements in Drosophila perform the entirely beneficial and crucial role for the host organism by maintaining telomere length but based on the data we see in this study we can speculate about another potential in their functioning, such as adaptive role against oxidative stress.

Acknowledgements:

We thank James Mason for critical reading of the manuscript and Marie Korchová for technical assistance. Peter Klepsatel is acknowledged for supervision of Tomáš Krůček during his research stay at the Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany. This work was supported by the grant no. 14-07172S from the Grant Agency of the Czech Republic, by the grant no. 052/2013/P and the grant no. 038/2014/P from the Grant Agency of the University of South Bohemia, by additional grants from the Grant Agency of the University of South Bohemia, and by the Max Planck Society. We acknowledge the use of research infrastructure that has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 316304. Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study.

References

Bednářová, A., Krishnan, N., Cheng, I.-C., Večeřa, J., Lee, H.-J., Kodrík, D., 2013. Adipokinetic hormone counteracts oxidative stress elicited in insects by hydrogen peroxide: in vivo and in vitro study. Physiol. Entomol. 38, 54–62.

Brand, A.H., Perrimon, N., 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401–15.

Calabrese, E.J., 2004. Hormesis: a revolution in toxicology, risk assessment and medicine. EMBO Rep. 5, S37.

Capkova Frydrychova, R., Biessmann, H., Konev, A.Y., Golubovsky, M.D., Johnson, J., Archer, T.K., Mason, J.M., 2007. Transcriptional activity of the telomeric retrotransposon HeT-A in Drosophila melanogaster is stimulated as a consequence of subterminal deficiencies at homologous and nonhomologous telomeres. Mol. Cell. Biol. 27, 4991–5001.

Capkova Frydrychova, R., Mason, J.M., Biessmann, H., 2009. Regulation of telomere length in Drosophila. Cytogenet. Genome Res. 122, 356–364.

Coluzzi, E., Colamartino, M., Cozzi, R., Leone, S., Meneghini, C., O'Callaghan, N., Sgura, A., 2014. Oxidative Stress Induces Persistent Telomeric DNA Damage Responsible for Nuclear Morphology Change in Mammalian Cells. PLoS One 9, e110963.

Cui, H., Kong, Y., Zhang, H., 2012. Oxidative stress, mitochondrial dysfunction, and aging. J. Signal Transduct. 2012, 646354.

Danilevskaya, O.N., Arkhipova, I.R., Traverse, K.L., Pardue, M.L., 1997. Promoting in tandem: the promoter for telomere transposon HeT-A and implications for the evolution of retroviral LTRs. Cell 88, 647–55.

Danilevskaya, O.N., Slot, F., Traverse, K.L., Hogan, N.C., Pardue, M.L., 1994. Drosophila telomere transposon HeT-A produces a transcript with tightly bound protein. Proc. Natl. Acad. Sci. U. S. A. 91, 6679–82.

Denchi, E.L., 2009. Give me a break: how telomeres suppress the DNA damage response. DNA Repair (Amst). 8, 1118–26.

George, J. a, Pardue, M.-L., 2003. The promoter of the heterochromatic Drosophila telomeric retrotransposon, HeT-A, is active when moved into euchromatic locations. Genetics 163, 625–35.

Houben, J.M.J., Moonen, H.J.J., van Schooten, F.J., Hageman, G.J., 2007. Chronic oxidative stress and telomere shortening, Chemico-Biological Interactions.

Chan, S.W., Blackburn, E.H., 2002. New ways not to make ends meet : telomerase , DNA damage proteins and heterochromatin. Oncogene 21, 553–63.

Choi, H.S., An, J.J., Kim, S.Y., Lee, S.H., Kim, D.W., Yoo, K.-Y., Won, M.H., Kang, T.-C., Kwon, H.J., Kang, J.H., Cho, S.-W., Kwon, O.-S., Park, J., Eum, W.S., Choi, S.Y., 2006. PEP-1-SOD fusion protein efficiently protects against paraquat-induced dopaminergic neuron damage in a Parkinson disease mouse model. Free Radic. Biol. Med. 41, 1058–68.

Krůček, T., Korandová, M., Šerý, M., Frydrychová, R.Č., Krůček, T., Korandová, M., Szakosová, K., 2015. Effect of low doses of herbicide paraquat on antioxidant defense in Drosophila. Arch. Insect Biochem. Physiol. 88, 235–48.

Martínez-Guitarte, J.L., Díez, J.L., Morcillo, G., 2008. Transcription and activation under environmental stress of the complex telomeric repeats of *Chironomus thummi*. Chromosome Res. 16, 1085–96.

Mason, J.M., Frydrychova, R.C., Biessmann, H., 2008. Drosophila telomeres: an exception providing new insights. Bioessays 30, 25–37.

Mason, J.M., Randall, T.A., Frydrychova, R.C., 2016. Telomerase lost□? Chromosoma 125, 65–73.

Mattson, M.P., 2008. Hormesis defined. Ageing Res. Rev. 7, 1–7.

Missirlis, F., Phillips, J.P., Jäckle, H., 2001. Cooperative action of antioxidant defense systems in Drosophila. Curr. Biol. 11, 1272–7.

Mollace, V., Iannone, M., Muscoli, C., Palma, E., Granato, T., Rispoli, V., Nisticò, R., Rotiroti, D., Salvemini, D., 2003. The role of oxidative stress in paraquat-induced neurotoxicity in rats: protection by non peptidyl superoxide dismutase mimetic. Neurosci. Lett. 335, 163–6.

Mourier, T., Nielsen, L.P., Hansen, A.J., Willerslev, E., 2014. Transposable elements in cancer as a by-product of stress-induced evolvability. Front. Genet. 5, 1–8.

Nandakumar, J., Cech, T.R., 2013. Finding the end: recruitment of telomerase to the telomere. Nat. Rev. Mol. Cell Biol. 14, 69–82.

Oikawa, S., Tada-Oikawa, S., Kawanishi, S., 2001. Site-specific DNA damage at the GGG sequence by UVA involves acceleration of telomere shortening. Biochemistry 40, 4763–4768.

Pardue, M.L., DeBaryshe, P.G., 1999. Drosophila telomeres: two transposable elements with important roles in chromosomes. Genetica 107, 189–96.

Pardue, M.-L., DeBaryshe, P.G., 2003. Retrotransposons provide an evolutionarily robust non-telomerase mechanism to maintain telomeres. Annu. Rev. Genet. 37, 485–511.

Passos, F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., Zglinicki, T. Von, 2007. Mitochondrial Dysfunction Accounts for the Stochastic Heterogeneity in Telomere-Dependent Senescence. Plos Biol. 5, 1138 –1151.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.

Večeřa, J., Krishnan, N., Alquicer, G., Kodrík, D., Socha, R., 2007. Adipokinetic hormone-induced enhancement of antioxidant capacity of Pyrrhocoris apterus hemolymph in response to oxidative stress. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 146, 336–42.

Velki, M., Kodrík, D., Večeřa, J., Hackenberger, B.K., Socha, R., 2011. Oxidative stress elicited by insecticides: a role for the adipokinetic hormone. Gen. Comp. Endocrinol. 172, 77–84.

von Zglinicki, T., 2002. Oxidative stress shortens telomeres. Trends Biochem. Sci. 27, 339–44.

Walter, M.F., Biessmann, H., 2004. Expression of the telomeric retrotransposon HeT-A in Drosophila melanogaster is correlated with cell proliferation. Dev. Genes Evol. 214, 211–9.

Wright, W.E., Piatyszek, M.A., Rainey, W.E., Byrd, W., Shay, J.W., 1996. Telomerase Activity inHuman Germline and Embryonic Tissues and Cells. Dev. Genet. 18, 173–179.

Zemanová, M., Stašková, T., Kodrík, D., 2016. Role of adipokinetic hormone and adenosine in the anti-stress response in Drosophila melanogaster. J. Insect Physiol. 92, 39–47.

Zhu, H., Belcher, M., van der Harst, P., 2011. Healthy aging and disease: role for telomere biology? Clin. Sci. (Lond). 120, 427–40.

Figures

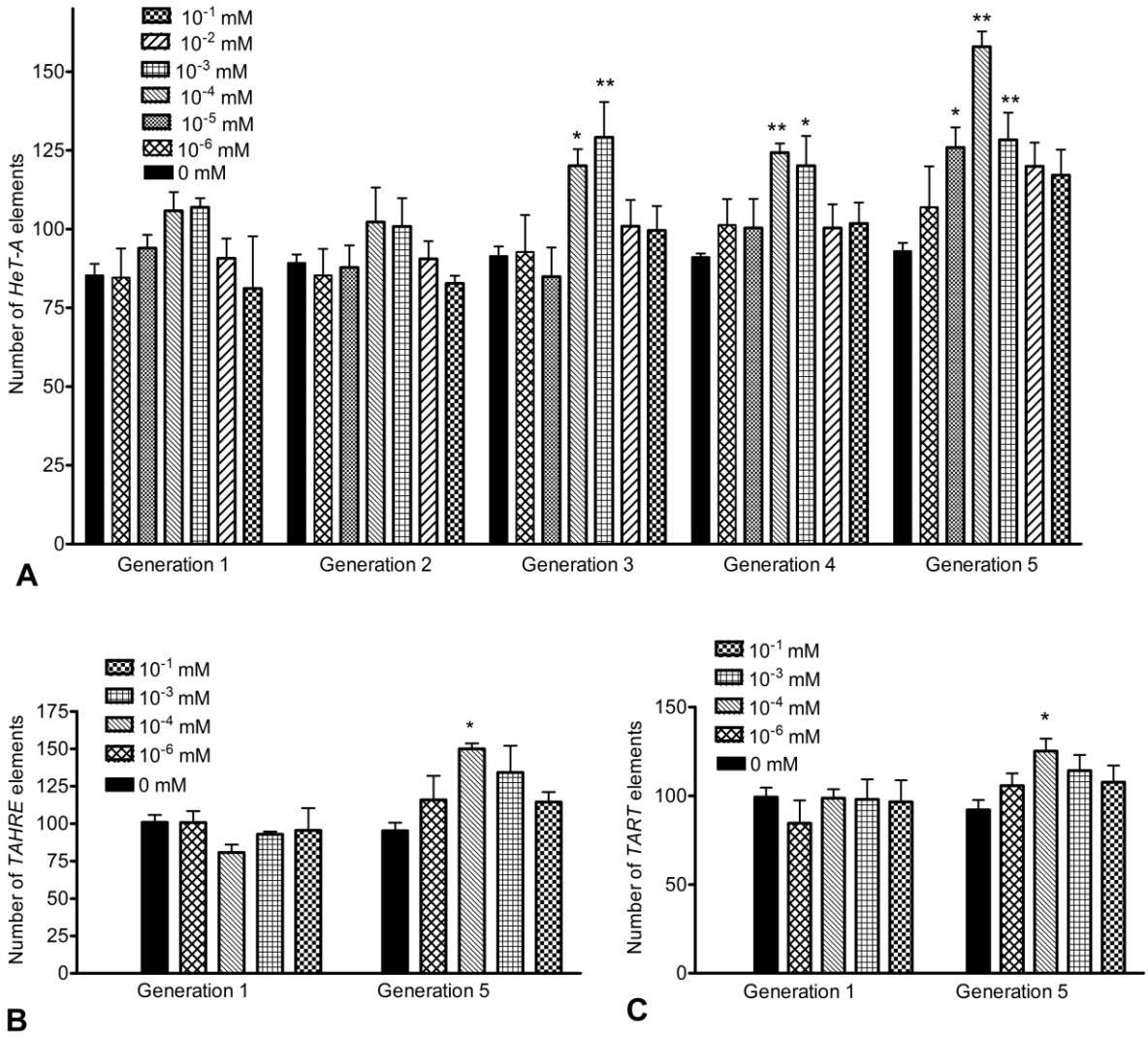

Fig. 1. Number of telomeric retroelements in genomes of tested flies. **A.** Number of *HeT-A* in five successive generations. Data were analyzed using two-way ANOVA analysis. Statistically significant differences were revealed with both generation ($P < 0.001$) and paraquat concentration ($P < 0.01$) as the independent factors. The analysis was followed by a Bonferroni posttest (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Number of *TART* (**B**) and *TAHRE* (**C**) in the 1st and 5th generation. Data were analyzed using one-way ANOVA analysis (*TAHRE*, $P < 0.05$; *TART*, no significant differences) followed by a Dunnett's Multiple Comparison Test (*$P < 0.05$). Error bars in the graph represent mean ± SEM of at least three independent experiments.

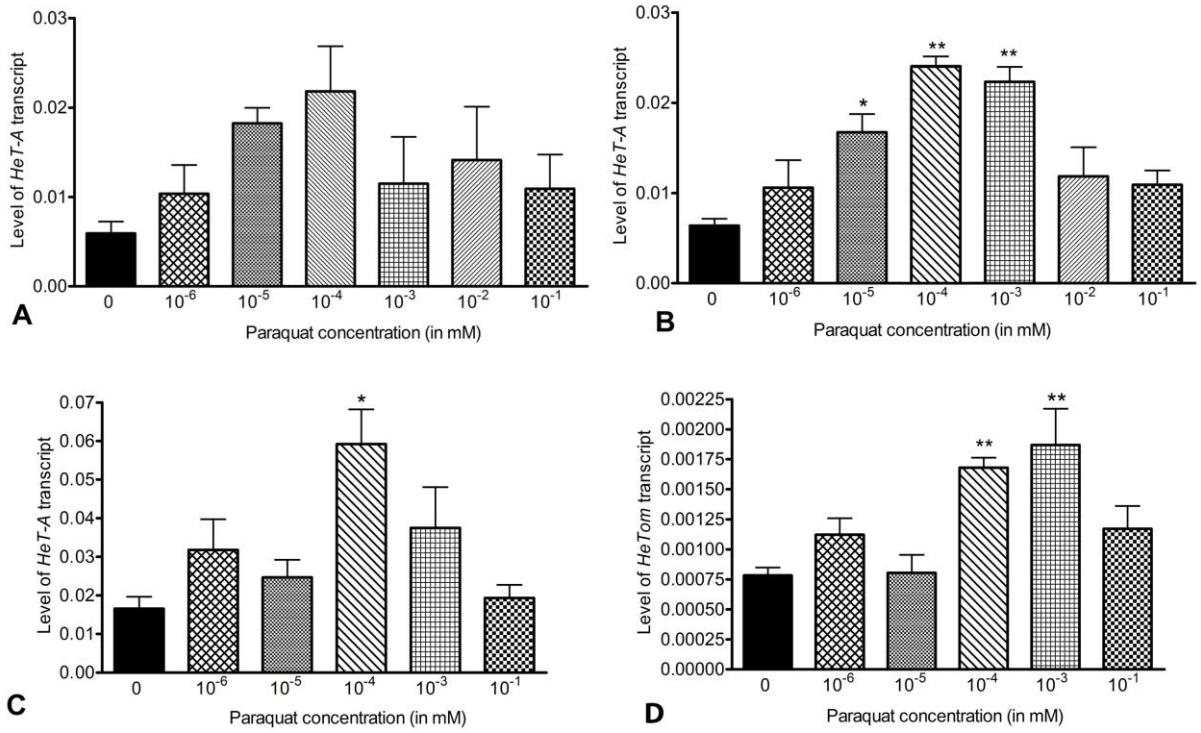

Fig. 2. Effect of paraquat on transcript levels. Transcript levels of *HeT-A* were measured in the first (**A**) and the fifth generation of Oregon R (**B**) and the first generation of y^1 w^{67c23}; *HeTom* line (**C**). Transcript levels of the *HeTom* transgene in y^1 w^{67c23}; *HeTom* line (**D**). The transcript levels were measured relative to *RpL32* transcripts. Data were analyzed using one-way ANOVA ($P < 0.001$) followed by a Dunnett's Multiple Comparison Test (*$P < 0.05$, **$P < 0.01$). Error bars in the graph represent mean ± SEM of five independent experiments.

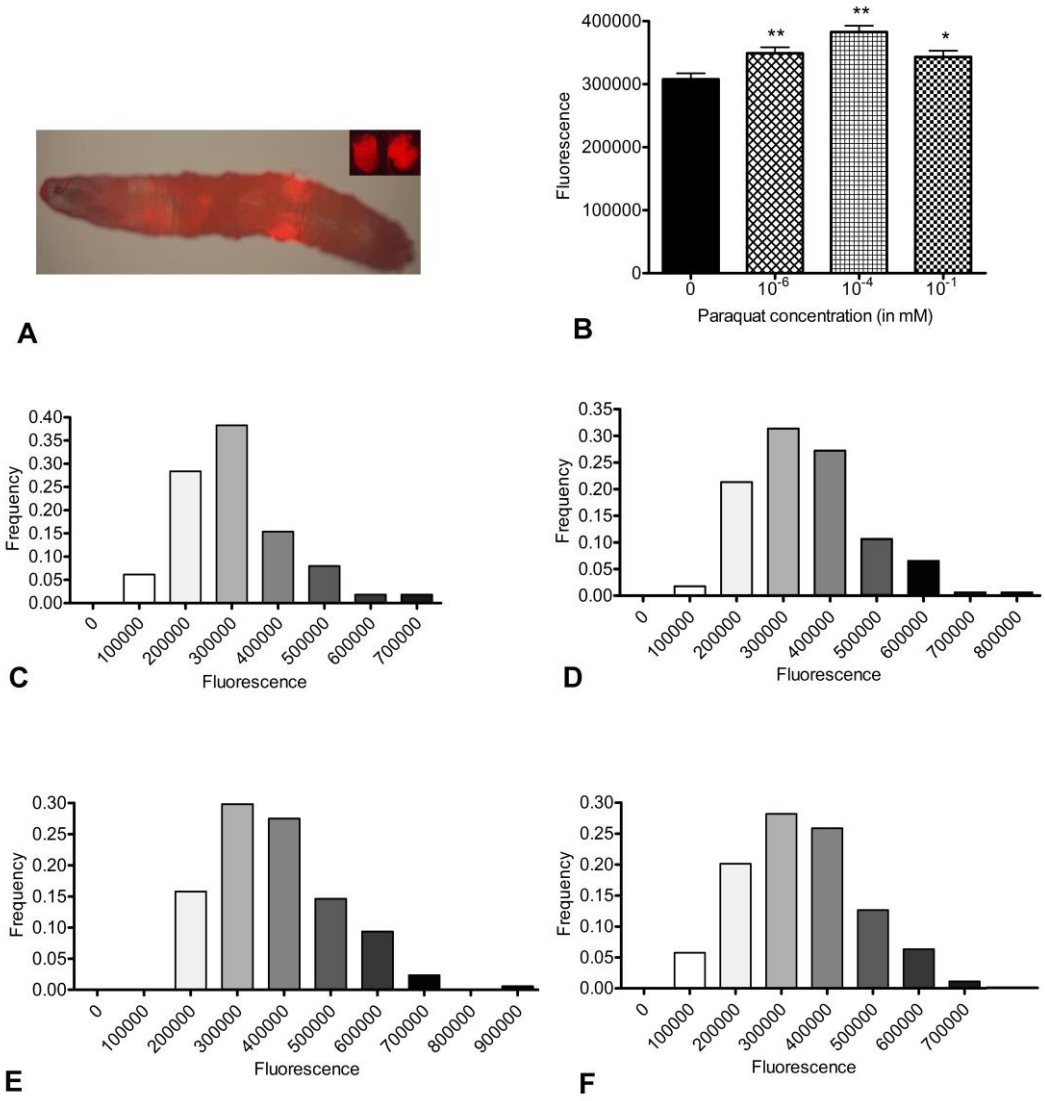

Fig. 3. *HeTom* expression. **A.** A bright field image of a 3rd instar male larva is merged with an image of the fluorescence signal from the same larva. Strong expression of *HeTom* is obvious in testes. Dissected testes are shown in upper right corner. **B.** Intensity of *HeTom* fluorescence recorded in dissected larval testes of the paraquat-treated and control flies. Data on intensity of fluorescence, recorded from testes of individual larvae, were divided into bins, where bin size was 10 000, and frequency distribution was calculated in control flies (**C**) and flies treated with paraquat: 10^{-6}mM (**D**), 10^{-4}mM (**E**), and 10^{-1}mM (**F**).

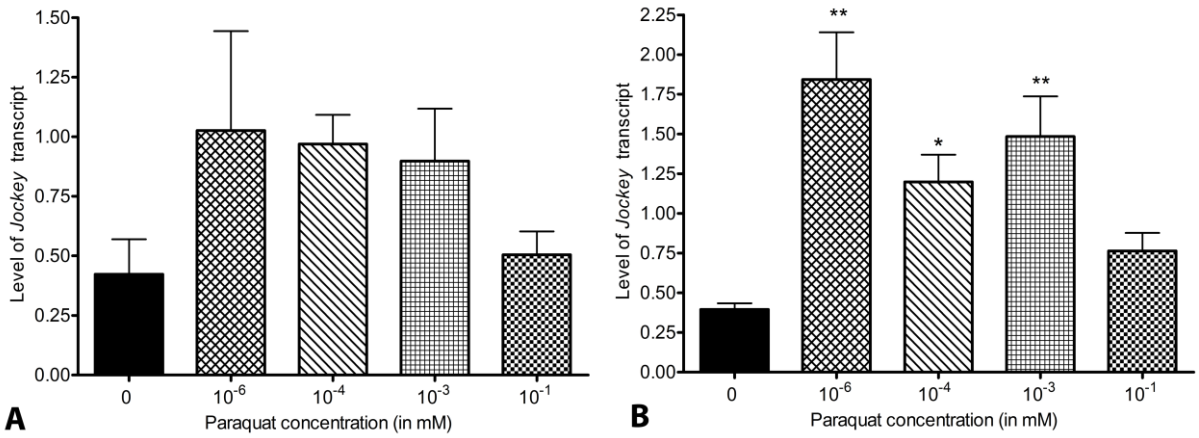

Fig. 4. Effect of paraquat on *Jockey* element in Oregon R. Transcript levels of *Jockey* relative to *RpL32* transcript were measured in the first (**A**) and in the fifth (**B**) generation of Oregon R. Data were analyzed using one-way ANOVA ($P < 0.001$) followed by a Dunnett's Multiple Comparison Test (*$P < 0.05$, **$P < 0.01$). Error bars in the graph represent mean ± SEM of five independent experiments.

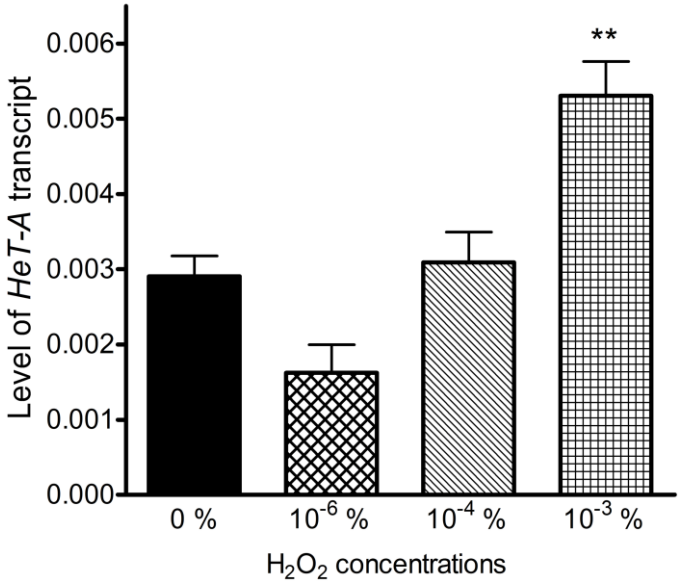

Fig. 5. Effect of hydrogen peroxide on transcript levels of *HeT-A* element. The transcript levels were measured relative to *RpL 32* transcript. Data were analyzed by one-way ANOVA ($P < 0.001$) followed by a Dunnett's Multiple Comparison Test (*$P < 0.01$). Error bars in the graph represent mean ± SEM of three independent experiments.

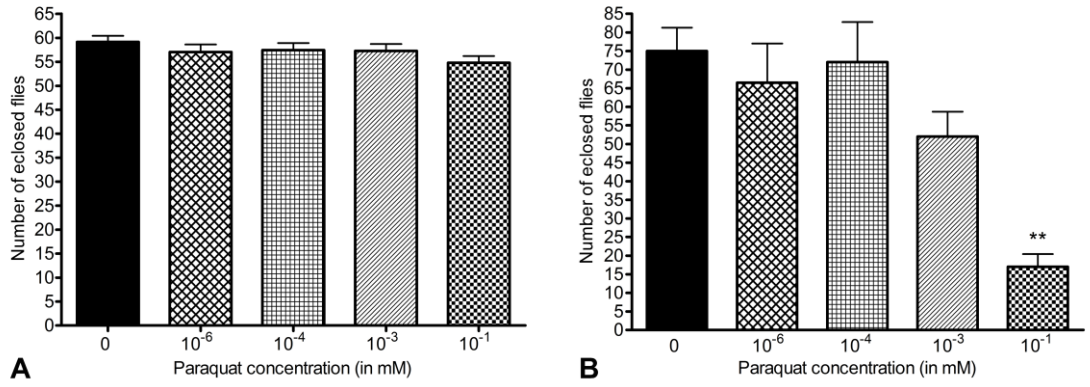

Fig. 6. Effect of paraquat on number of eclosed flies. **A.** Number of flies eclosed from eighty eggs, which were collected from the tested groups and transferred into fresh vials. **B.** Number of flies eclosed from all eggs laid by ten females in a vial. Error bars in the graphs represent mean ± SEM of at least four independent experiments. Data were analyzed by one-way ANOVA ($P < 0.001$) followed by a Dunnett's Multiple Comparison Test (*$P < 0.05$, **$P < 0.01$).

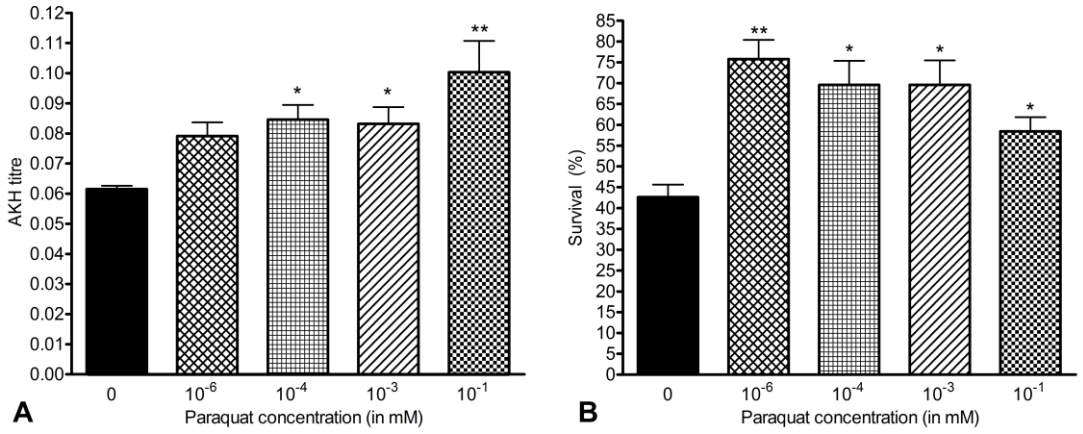

Fig. 7. Resistance against oxidative stress after acute exposure to a high dose of paraquat. For five generations, Oregon R line was treated with the various paraquat concentrations. Then, adult progeny of the fifth generation was exposed to 20 mM paraquat for 24 hours, and the AKH titre (**A**) and the survival rate (**B**) was evaluated. Data were analyzed by one-way ANOVA ($P < 0.01$) followed by a Dunnett's Multiple Comparison Test (*$P < 0.05$, **$P < 0.01$). Error bars in the graph represent mean ± SEM of three independent experiments.

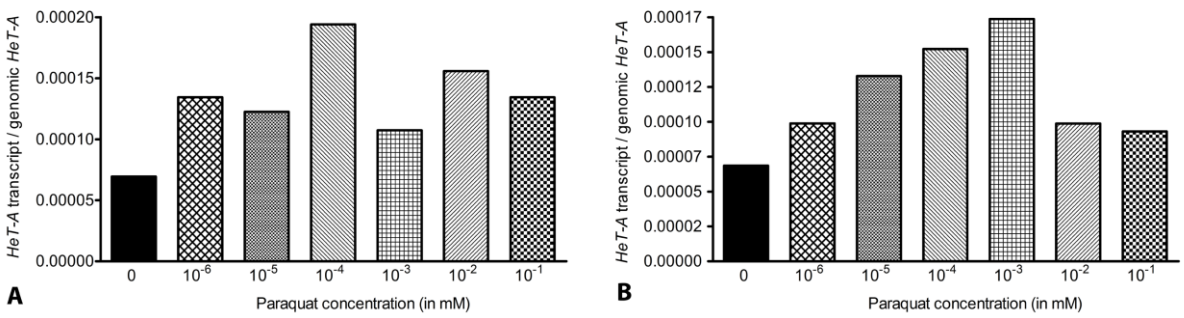

Fig. S1. The ratio between *HeT-A* transcript level and number of genomic *HeT-A.* The ratio between *HeT-A* transcript level and number of genomic *HeT-A* was calculated in the 1st (**A**) and 5th (**B**) generation based on the data presented in **Fig. 1A** and **Fig. 2A**, **B**.

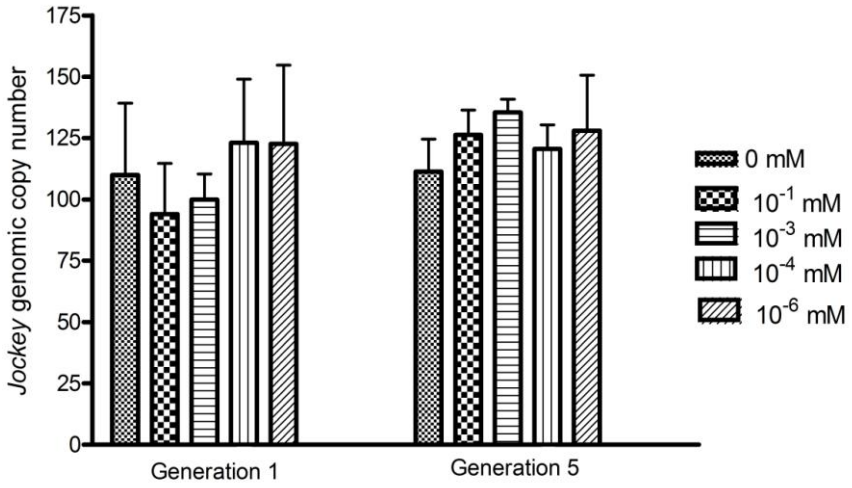

Fig. S2. Number of non-telomeric retroelement *Jockey* in genomes of tested flies. Genomic copy numbers of *Jockey* were measured in the 1st and 5th generations. Error bars in the graph represent mean ± SEM of four independent experiments.

5. SUMMARY

Telomerase activity is considered as the most common mechanism to compensate telomere shortening, playing a role in regulation of cell proliferation and aging processes. By preventing premature telomere shortening telomerase acts as a determinant of organismal lifespan, as documented by both telomerase-deficient mice and by mutations in telomerase components in humans that causes accelerated telomere attrition leading to premature adult stem cell dysfunction and accelerated aging (Blasco et al. 1997; Herrera et al. 1999; Tsakiri et al. 2007; Armanios et al. 2007). Most research on telomerase activity and telomere length has been performed on vertebrates.

With a primarily focus on the activity of telomere maintenance mechanisms in insects this PhD thesis proved that telomerase that is specific to the TTAGG telomeric sequence is the most common telomere maintenance in insect and that, similarly to vertebrates, insect telomerase is developmentally regulated. The highest activity of telomerase was found in embryos and gonads in both holometabolous and hemimetabolous insects. The decline of telomerase activity was observed during development of *P. americana* as the representative of holometabolous insect, where immature forms gradually increase in size before they become adults, which resembles telomerase decline with development and age of vertebrates (Wright et al. 1996; Hornsby 2007).

Different species have different life history strategies in terms of different allocation of resources among growth, reproduction, and self-maintenance. One of important trades-off in living systems is the negative relationship between lifespan and growth within species, and telomeres and oxidative stress are considered to play a role in this relationship (Monaghan 2010; Haussmann & Marchetto 2010). Age-related decline in telomere length is well established, and one might expect that particularly long-lived species would have relatively long telomeres. However, no link was observed between telomere length and an average lifespan of numerous vertebrate species. For instance, mice are short-lived animals compared to humans although they have longer telomeres and most somatic cells of adult mice have telomerase activity. Also, when compared humans to closely related primates, humans have relatively short telomeres (Blasco 2005; Hemann & Greider 2000; Kipling & Cooke 1990; Kakuo et al. 1999; Seluanov et al. 2007). Telomere attrition resulting from the insufficient

telomerase activity may be a factor in determining some age-related characteristics in humans, yet telomerase is probably not a factor in determining the differences in aging rate among species (Hornsby 2007). Instead, telomerase activity in vertebrates seems to be co-evolved with body mass. The comparison of large and small mammals reveals that large mammals appear to repress telomerase activity in their somatic cells during adult age to a greater extent than small mammals do (Seluanov et al. 2007). For instance, while telomerase is active in most of somatic cells of adult mice, most somatic cells of large mammals, such as primates, sheep or horses, show a decline or absence of telomerase. It is thought that replicative senescence have developed as an adaptive anti-tumor mechanism (Campisi 2001) and that large species, which are relatively long-lived species, could experience a greater susceptibility to cancer compared to small species because of their higher number of cells (Nunney 1999). Therefore, large species might evolve repression of telomerase activity as the anti-cancer mechanism. Regarding trades-off strategies, a decline of telomerase activity can act as a tumor protective mechanism, but on the other hand it also accelerates cellular senescence.

We tested telomere length and telomerase activity in long- and short-lived honey bee queens and honey bee workers, respectively. Consistently with the previous observation in ant *Lasius niger*, where telomere length was not found to be different when compared between long-lived queens and short-lived workers (Jemielity at al. 2007), no differences in telomere length have been observed between workers and queens of honey bees. Therefore, we can conclude that telomere length does not seem to be a determining factor in lifespan of social insects. However, when we tested telomerase activity we found its elevated levels in the brains and fat body of queens leading us to speculate that the up-regulation of telomerase activity in certain tissues of queens might contribute to the extended lifespan or extraordinary reproduction of honey bee queens, and perhaps explaining the contradiction to cost of reproduction phenomenon observed in social insects. There is emerging evidence that lack of telomerase increases oxidative stress and decreases neuronal survival and, thus, telomerase has a beneficial role on human brain (Saretzki 2016). But it is still too early to speculate what is the exact role of telomerase in honey bee queen brains as well as fat body.

Telomere attrition and oxidative stress are two separate aging mechanisms at cellular and organismal level, yet telomere attrition is tightly connected to oxidative stress as telomere

length is determined by both telomere maintenance mechanisms and telomere loss events such as the incomplete DNA replication and oxidative damage. So far, free oxygen radicals were believed to act as cardinal factors in acceleration of telomere shortening, and therefore, in hastening of cellular and organismal aging. This PhD thesis shows a novel phenomenon where oxygen free radicals may act in terms of telomere lengthening rather than shortening.

6. REFERENCES

- Abad JP, de Pablos B, Osoegawa K, de Jong PJ, Martín-Gallardo A,Villasante A (2004). *TAHRE*, a novel telomeric retrotransposon from *Drosophila melanogaster*, reveals the origin of *Drosophila* telomeres. Molecular Biology and Evolution 21(9): 1620-1624.

- Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A (2004). Pesticides and oxidative stress: a review. International medical Journal of Experimental and Clinical Research 10(6): 141-147.

- Amdam GV, Norberg N, Omholt SW, Kryger P, Lourenço AP, Bitondi MMG, Simões ZLP (2005). Higher vitellogenin concentrations in honey bee workers may be an adaptation to life in temperate climates. Insect Sociaux 52: 316-319.

- Amdam GV, Omholt SW (2003). The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. Journal of Theoretical Biology 223: 451-464.

- Amdam GV, Simões ZLP, Hagen A, Norberg K, Schrøder K, Mikkelsen Ø, Kirkwood TBL, Omholt SW (2004). Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. Experimental Gerontology 39: 767-773.

- Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA III, Lansdorp PM, Greider CW, Loyd JE (2007). Telomerase mutations in families with idiopathic pulmonary fibrosis. The New Englend Journal of Medicine 356: 1317-1326.

- Aronstein KA, Hayes GW (2004). Antimicrobial activity of allicin against honey bee pathogens. Journal of Apicultural Research 43: 57-59.

- Arrese EL, Soulages J (2010). Insect fat body: energy, metabolism, and regulation. Annual Review of Entomology 55: 207-225.

- Barnham KJ, Masters CL & Bush AI (2004). Neurodegenerative diseases and oxidative stress. Nature Reviews Drug Discovery 3(3): 205-214.

- Biessmann H & Mason JM (2003). Telomerase-independent mechanisms of telomere elongation. Cellular and Molecular Life Sciences 60(11): 2325-2333.
- Biessmann H, Champion LE, O'Hair M, Ikenaga K, Kasravi B, Mason JM (1992). Frequent transpositions of *Drosophila melanogaster* HeT-A transposable elements to receding chromosome ends. EMBO J 11(12): 4459-4469.
- Biessmann H, Mason JM (2003). Telomerase-independent mechanisms of telomere elongation. Cellular and Molecular Life Sciences 60: 2325-2333.
- Biessmann H, Mason JM, Ferry K, Dhulst M, Valgeirsdottir K, Traverse KL, Pardue ML (1990). Addition of Telomere-Associated HeT Dna-Sequences “Heals“ Broken Chromosome Ends in Drosophila. Cell 61(4): 663-673.
- Biessmann H, Valgeirsdottir K, Lofsky A, Chin C, Ginther B, Levis RW & Pardue ML (1992). *HeT-A*, a transposable element specifically involved in "healing" broken chromosome ends in *Drosophila melanogaster*. Molecular and Cellular Biology 12(9): 3910-3918.
- Bilikova K, Popova M, Trusheva B, Bankova V (2013). New anti-Paenibacillus larvae substances purified from propolis. Apidologie 44: 278-285.
- Blackburn EH (1991). Structure and function of telomeres. Nature 350 (6319): 569-573.
- Blagosklonny MV (2010). Revisiting the antagonistic pleiotropy theory of aging: TOR-driven program and quasi-program. Cell Cycle 9(16): 3171-3176.
- Blasco MA (2005). Mice with bad ends: mouse models for the study of telomeres and telomerase in cancer and aging. EMBO Journal 24:1095-1103.
- Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW (1997). Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 91: 25-34.
- Blasco MA, Lee HW, Rizen M, Hanahan D, DePinho R, Greider CW (1997). Mouse models for the study of telomerase. Ciba Foundation Symposium 211: 160-170.

- Brand AH, Perrimon N (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118: 401-415.
- Brodschneider R, Crailsheim K (2010). Nutrition and health in honey bees. Apidologie 41: 278-294.
- Budanov AV, Karin M (2008). p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell. 2008 134(3): 451-460.
- Buttstedt A, Ihling CH, Pietzsch M, Moritz RFA, (2016). Royalactin is not a royal making of a queen. Nature 537: E10-E12.
- Buttstedt A, Moritz RFA, Erler S (2014). Origin and function of the major royal jelly proteins of the honeybee (*Apis mellifera*) as members of the yellow gene family. Biological Reviews 89: 255-269.
- Cadenas E, Davies KJ (2000). Mitochondrial free radical generation, oxidative stress, and aging. Free Radical Biology and Medicine 29(3): 222-230.
- Cadenas E, Sies H (1998). The lag phase. Free Radical Research 28(6): 601-609.
- Calabrese EJ (2004). Hormesis: from marginalization to mainstream: a case for hormesis as the default dose-response model in risk assessment. Toxicology and Applied Pharmacology 197(2): 125-136.
- Campisi J (2001). Cellular senescence as a tumor-suppressor mechanism. Trends in Cell Biology 11: S27–S31.
- Capkova Frydrychova R, Biessmann H, Konev AY, Golubovsky MD, Johnson J, Archer TK, Mason JM (2007). Transcriptional activity of the telomeric retrotransposon *HeT-A* in *Drosophila melanogaster* is stimulated as a consequence of subterminal deficiencies at homologous and nonhomologous telomeres. Molecular and Cellular Biology 27: 4991-5001.
- Capkova Frydrychova R, Mason JM (2013). Telomeres: Their structure and maintenance. David Stuart (ed.). The Mechanisms of DNA Replication. Intech, Open Access Publisher, Rijeka, Croatia, pp. 423-443.

- Capkova Frydrychova R, Mason JM, Biessmann H (2009). Regulation of telomere length in *Drosophila*. Cytogenetic and Genome Research 122: 356-364.
- Casacuberta E, Pardue ML (2005). *HeT-A* and *TART*, two *Drosophila* retrotransposons with a bona fide role in chromosome structure for more than 60 million years. Cytogenetic and Genome Research 110: 152-159.
- Cenci G, Ciapponi L, Gatti M (2005). The mechanism of telomere protection: a comparison between *Drosophila* and humans. Chromosoma 114: 135-145.
- Chan SW, Blackburn EH (2002). New ways not to make ends meet: telomerase, DNA damage proteins and heterochromatin. Oncogene 21: 553-563.
- Chance B, Sies H, Boveris A (1979). Hydroperoxide Metabolism in Mammalian Organs. Physiological Reviews 59(3): 527-605.
- Chen D, Thomas EL, Kapahi P (2009). HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in Caenorhabditis elegans. PLoS Genetics 5(5): e1000486.
- Chen QM, Bartholomew JC, Campisi J, Acosta M, Reagan JD, Ames BN (1998). Molecular analysis of H_2O_2-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. Biochemical Journal 332(1): 43-50.
- Cheng SE, Lee IT, Lin CC, Kou YR, Yang CM (2010). Cigarette smoke particle-phase extract induces HO-1 expression in human tracheal smooth muscle cells: role of the c-Src/NADPH oxidase/MAPK/Nrf2 signaling pathway. Free Radical Biology and Medicine 48(10): 1410-1422.
- Choi HS, An JJ, Kim SY, Lee SH, Kim DW, Yoo KY, Won MH, Kang TC, Kwon HJ, Kang JH, Cho SW, Kwon OS, Park J, Eum WS, Choi SY (2006). PEP-1-SOD fusion protein efficiently protects against paraquat-induced dopaminergic neuron damage in a Parkinson disease mouse model. Free Radical Biology and Medicine 41(7):1058-1068.
- Cooper GM (2000). DNA replication. The Cell: A Molecular Approach.

- Corona M, Velarde RA, Remolina S, Moran-Lauter A, Wang Y, Hughes KA, Robinson GE (2007). Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. PNAS (104) 17: 7128-7133.

- Damjanovic AK, Yang Y, Glaser R, Kiecolt-Glaser JK, Nguyen H, Laskowski B, Zou Y, Beversdorf DQ, Weng NP (2007). Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. Journal of Immunology 179: 4249-4254.

- Danilevskaya ON, Arkhipova IR, Pardue ML, Traverse KL (1997). Promoting in tandem: the promoter for telomere transposon *HeT-A* and implications for the evolution of retroviral LTRs. Cell 88(5): 647-655.

- Danilevskaya ON, Slot Treverse KL, Hogan NC, Pardue M-L (1994). *Drosophila* telomere transposon HeT-A produces a transcript with tightly bound protein. Proceedings of the National Academy of Sciences of the USA 91: 6679-3382.

- Danilevskaya ON, Traverse KL, Hogan NC, DeByryshe PG, Pardue ML (1999). The two *Drosophila* telomeric transposable elements have very different patterns of transcription. Molecular and Cellular Biology 19: 873-881.

- Denchi EL (2009). Give me a break: how telomeres suppress the DNA damage response. DNA Repair 8: 1118-1126.

- Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H (2002). Free radical-induced damage to DNA: mechanisms and measurement. Free radical biology and medicine 32: 1102-1115.

- Entringer S, Epel ES, Kumsta R, Lin J, Hellhammer DH, Blackburn EH, Wüst S, Wadhwa PD (2011). Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. Proceedings of National Academy of Sciences of the USA 108: E513-E518.

- Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM (2004). Accelerated telomere shortening in response to life stress. Proceedings of the National Academy of Sciences U S A 101(49): 17312-17315.

- Epel ES, Lin J, Dhabhar FS, Wolkowitz OM, Puterman E, Karan L, Blackburn EH (2010). Dynamics of telomerase activity in response to acute psychological stress. Brain Behavior and Immunity 24: 531-539.

- Eremia N, Bahcivanji M, Zagareanu A. (2013). Study of Influence of Algal „*Chlorella vulgaris*“ Suspencion on Grouth and Productivity of Bees Families. University of Agricultural Sciences and Veterinary Medicine Iasi, Lucrări Ştiinţifice-Seria Zootehnie, vol. 59: 148 - 152.

- Ermak G, Davies KJA (2002). Calcium and oxidative stress: from cell signaling to cell death. Molecular Immunology (10): 713-721.

- Fajkus J, Sykorova E, Leitch AR (2005). Telomeres in evolution and evolution of telomeres. Chromosome Research 13(5): 469-479.

- Flanders SE (1960). Caste in the honey bee. Insectes Sociaux 7: 9-16.

- Flores I, Benetti R, Blasco MA (2006). Telomerase regulation and stem cell behaviour. Current Opinion in Cell Biology 18 (3): 254-260.

- Formesyn EM, Cardoen D, Ernst UR, Danneels EL, van Vaerenbergh M, de Koker D, Verleyen P, Wenseleers T, Schoofs L, de Graaf DC (2014). Reproduction of honeybee workers is regulated by epidermal growth factor receptor signaling. General and Comparative Endocrinology 197 (C): 1-4.

- Forsyth NR, Wright WE, Shay JW (2002). Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again. Differentiation 69: 188-197.

- Frydrychova R, Grossmann P, Trubac P, Vitkova M, Marec F (2004). Phylogenetic distribution of TTAGG telomeric repeats in insects. Genome 47:163-178.

- Frydrychova R, Marec F (2002). Repeated losses of TTAGG telomere repeats in evolution of beetles *(Coleoptera)*. Genetica 115: 179-187.

- Fujiwara H, Osanai M, Matsumoto T, Kojima KK (2005). Telomere-specific non-LTR retrotransposons and telomere maintenance in the silkworm, *Bombyx mori*. Chromosome Research 13 (5): 455-467.

- George J, Pardue ML (2003). The promoter of the heterochromatic *Drosophila* telomeric retrotransposon, *HeT-A*, is active when moved into euchromatic locations. Genetics 163: 625-635.

- Geserick C, Blasco MA (2006). Novel roles for telomerase in aging. Mechanisms of Ageing and Development 127(6): 579-583.

- Gkogkolou P, Böhm M (2012). Advanced glycation end products; Key players in skin aging? Dermato-Endocrinology 4 (3): 259-270.

- Gomes NMV, Shay JW, Wright WE (2011). Telomere Biology in Metazoa. Federation of European Biochemical Societies 584 (17): 3741-3751.

- Griendling KK & FitzGerald GA (2003). Oxidative stress and cardiovascular injury part I: basic mechanisms and in vivo monitoring of ROS. Circulation 108 (16): 1912-1916.

- Grigolava IV, Ksenzenko M, Konstantinob AA, Tikhonov AN, Kerimov TM (1980). Tiron as a spin-trap for superoxide radicals produced by the respiratory chain of submitochondrial particles. Biokhimiia (45): 75-82.

- Guarente A (2007). Sirtuins in Aging and Disease. Cold Spring Harbor Symposia on Quantitative Biology 72: 483-488.

- Guidugli KR, Nascimento AM, Amdam GV, Barchuk AR, Omholt S, Simões ZLP, Hartfelder K (2005). Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. FEBS Letters 579: 4961-4965.

- Guo XM, Allen SK (1997). Fluorescence in situ hybridization of vertebrate telomere sequence to chromosome ends of the Pacific oyster, Crassostrea gigas Thunberg. Journal of Shellfish Research 16(1): 87-89.

- Hansen M, Boesen A, Holm L, Flyvbjerg A, Langberg H, Kjaer M (2013). Local administration of insulin-like growth factor-I (IGF-I) stimulates tendon collagen synthesis in humans. Scandinavian Journal of Medicine & Science in Sports 23(5): 614-619.
- Harley CB, Villeponteau B (1995). Telomeres and telomerase in aging and cancer. Current Opinion in Genetics and Development 5(2): 249-255.
- Harman D (1956). Aging: a theory based on free radical and radiation chemistry. The Journal of Gerontology 11: 298-300.
- Harman D (1972). The biologic clock: the mitochondria? Journal of the American Geriatrics Society 20(4):145-147.
- Harshman LG, Zera AJ (2007). The cost of reproduction: the devil in the details. Trends Ecology Evolution 22: 80-86.
- Hattori N, Nomoto H, Fukumitsu H, Mishima S, Furukawa S (2007). Royal jelly-induced neurite outgrowth from rat pheochromocytoma PC12 cells requires integrin signal independent of activation of extracellular signal-regulated kinases. Biomedical Research 28(3): 139-146.
- Haussmann MF, Marchetto NM (2010). Telomeres: Linking stress and survival, ecology and evolution. Current Zoology 56 (6): 714-727.
- Havukainen H, Münch D, Baumann A, Zhong S, Halskau Ø, Krogsgaard M, Amdam GV (2013). Vitellogenin Recognizes Cell Damage through Membrane Binding and Shields Living Cells from Reactive Oxygen. The Journal of Biological Chemistry 288(39): 28369-28381.
- Hayflick L, Moorhead PS (1961). The serial cultivation of human diploid cell strains. Experimental Cell Research 25: 585-621.
- Heidinger BJ, Blount JD, Boner W, Griffiths K, Metcalfe NB & Monaghan P (2012). Telomere length in early life predicts lifespan. The National Academy of Sciences 109(5): 1743-1748.

- Hemann MT, Greider CW (2000). Wild-derived inbred mouse strains have short telomeres. Nucleic Acids Research 28: 4474–4478.
- Herrera E, Samper E, Martin-Caballero J, Flores JM, Lee HW, Blasco MA (1999). Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. EMBO Journal 18: 2950-2960
- Honda Y, Fujita Y, Maruyama H, Araki Y, Ichihara K, Sato A, Kojima T, Tanaka M, Nozawa Y, Ito M, Honda S (2011). Lifespan-Extending Effects of Royal Jelly and Its Related Substances on the Nematode Caenorhabditis elegant. PLoS ONE 6(8): e23527.
- Hornsby PJ (2007). Telomerase and the aging process. Experimental Gerontology 42(7): 575-581.
- Houben JMJ, Moonen HJJ, van Schooten FJ, Hageman GJ (2008). Telomere length assessment: biomarker of chronic oxidative stress? Free Radical Biology and Medicine 44(3): 235-246.
- Inoue S, Koya-Miyata S, Ushio S, Iwaki K, Ikeda M, Kurimoto M (2003). Royal Jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage. Experimental Gerontology 38(9): 965-969.
- Jemielity S, Kimura M, Parker KM, Parker JD, Cao XJ, Aviv A, Keller L (2007). Short telomeres in short-lived males: What are the molecular and evolutionary causes? Aging Cell 6: 225–233.
- Johnson BR (2010). Division of labor in honeybees: form, function, and proximate mechanisms. Behavioral Ecology Sociobiology 64: 305-316.
- Kakuo S, Asaoka K, Ide T (1999). Human is a unique species among primates in terms of telomere length. Biochemical and Biophysical Research Communications 263: 308-314.
- Kamakura M (2011). Royalactin induces queen differentiation in honeybees. Nature 473: 478-483.

- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994). Specific association of human telomerase aktivity 965 with immortal cells and cancer. Science 266: 2011-2015.
- Kipling D, Cooke HJ (1990). Hypervariable ultra-long telomeres in mice. Nature 347: 400–402.
- Kirkwood TBL (2005). Understanding the Odd Science of Aging. Cell 120: 437-447.
- Klapper W, Kühne K, Singh KK, Heidorn K, Parwaresch R, Krupp G (1998). Longevity of lobsters is linked to ubiquitous telomerase expression. FEBS Letters 439(1-2): 143-146.
- Klaunig JE, Kamendulis LM & Hocevar BA (2010). Oxidative stress and oxidative damage in carcinogenesis. Toxicologic Pathology 38(1): 96-109.
- Kofman AE, McGraw MR, Payne CJ (2012). Rapamycin increases oxidative stress response gene expression in adult stem cells. Aging 4(4): 279-289.
- Korandova M, Frydrychova RC (2016). Activity of telomerase and telomeric length in *Apis mellifera*. Chromosoma 125: 405-411.
- Korb J (2016). Why do social insect queens live so long? Approaches to unravel the sociality-aging puzzle. Current Opinion in Insect Science 16: 104-107.
- Kotrschal A, Ilmonen P, Penn DJ (2007). Stress impacts telomere dynamics. Biology Letters 3: 128-130.
- Kunlin J (2010). Modern Biological Theories of Aging. Aging and Disease 1(2): 72-74.
- Lewis DFV, Eddershaw PJ, Dickens M, Tarbit MH, Goldfarb PS (1998). Structural determinants of cytochrome P450 substrate specificity, binding affinity and catalytic rate. Chemico-Biological Interactions 115(3): 175-199.
- Lin J, Epel E, Blackburn E (2008). Telomeres and Telomerase in Human Health and Disease. In: Gage F, Christen Y (eds) Retrotransposition, Diversity and the Brain. Research and Perspectives in Neurosciences. Springer, Berlin, Heidelberg.

- Lin J, Epel E, Blackburn E (2012). Telomeres and lifestyle factors: roles in cellular aging. Mutation Research 730: 85-89.

- Linger BR, Price CM (2010). Conservation of Telomere protein complexes: Shuffling through Evolution. National Institutes of Health Public Access 44: 434-446.

- Liu T, Tannergård P, Hackman P, Rubio C, Kressner U, Lindmark G, Hellgren D, Lambert B, Lindblom A (1999). Missense mutations in hMLH1 associated with colorectal cancer. Human Genetics 105(5):437-41.

- Loft S, Poulsen HE (1996). Cancer risk and oxidative DNA damage in man. Journal of Molecular Medicine 74(6): 297-312.

- Lourenço AP, Mackert A, Cristino ADS, Simões ZLP (2008). Validation of reference genes for gene expression studies in the honey bee, *Apis mellifera*, by quantitative real-time RT-PCR. Apidologie, Springer Verlag 39 (3): 372-385.

- Makarov VL, Hirose Y, Langmore JP (1997). Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. Cell 88: 657-666.

- Maritim AC, Sanders RA, Watkins JB 3rd. (2003). Diabetes, oxidative stress, and antioxidants: a review. Journal of Biochemical and Molecular Toxicology 17(1): 24-38.

- Martínez-Guitarte JL, Díez JL, Morcillo G (2008). Transcription and activation under environmental stress of the complex telomeric repeats of *Chironomus thummi*. Chromosome Research 16: 1085-1096.

- Martins R, Lithgow GJ, Link W (2016). Long live FOXO: unraveling the role of FOXO proteins in aging and longevity. Aging Cell 15: 196-207.

- Mason J, Capkova Frydrychova R, Biessmann H (2008). Drosophila telomeres: an exception providing new insights. BioEssays 30: 25-37.

- Mason JM, Randall TA, Capkova Frydrychova R (2016). Telomerase lost? Chromosoma 125(1): 65-73

- Mattson MP (2008). Hormesis defined. Ageing Research Reviews 7(1): 1-7.
- McEachern MJ, Blackburn EH (1994). A conserved sequence motif within the exceptionally diverse telomeric sequences of budding yeasts. Proceedings of the National Academy of Science USA 91: 3453-3457.
- Metcalfe NB, Alonso-Alvarez C (2010). Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Functional Ecology 24: 984-996.
- Meyne J, Ratliff RL, Moyzis RK (1989). Conservation of the human telomere sequence (TTAGGG)n among vertebrates. Proceedings of the National Academy of Sciences 86(18): 7049-7053.
- Mollace V, Iannone M, Muscoli C, Palma E, Granato T, Rispoli V, Nisticò R, Rotiroti D, Salvemini D (2003). The role of oxidative stress in paraquat-induced neurotoxicity in rats: protection by non peptidyl superoxide dismutase mimetic. Neuroscience Letters 335(3):163-166.
- Monaghan P (2010). Telomeres and life histories: the long and the short of it. Annals of New York Academy of Science 1206:130-142.
- Monathan P (2010). Telomeres and life histories: the long and the short of it. Annals of the New York Academy of Sciences 1206:130-142.
- Monroy Kuhn JM, Korb J (2016). Editorial overview: Social insects: aging and the re-shaping of the fecundity/longevity trade-off with sociality. Current Opinion in Insect Science 16: vii-x.
- Mutti NS, Dolezal AG, Wolschin F, Mutti JS, Gill KS, Amdam GV (2011). IRS and TOR nutrient-signaling pathways act *via* juvenile hormone to influence honey bee caste fate. The Journal of Experimental Biology 214: 3977-3984.
- Nasir NFM, Kannan TP, Sulaimann SA, Shamsuddin S, Ahmad A, Stangaciu S (2014). Telomeres and oxidative stress. British Journal of Medicine & Medical Research 4: 57-67.

- Nunney L (1999). Lineage selection and the evolution of multistage carcinogenesis. Proceeding of Biologycal Science 266: 493–498.
- Oikawa S, Kawanishi S (1999). Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. FEBS Letters 453(3): 365-368.
- Oikawa S, Tada-Oikawa S, Kawanishi S (2001). Site-specific DNA damage at the GGG sequence by UV involves acceleration of telomere shortening. Biochemistry 40(15): 4763-4768.
- Olovnikov AM (1973). A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. Journal of Theoretical Biology 41(1): 181-90.
- Ortega-Molina A, Serrano M (2013). PTEN in cancer, metabolism and aging. Trends in Endocrinology and Metabolism 24(4): 184-189.
- Ott M, Gogvadze V, Orrenius S, Zhivotovsky B (2007). Mitochondria, oxidative stress and cell death. Apoptosis 12(5): 913-922.
- Page RE Jr, Peng CY-S (2001). Aging and development in social insects with emphasis on the honey bee, *Apis mellifera L*. Experimental Gerontology 36(36): 695-711.
- Pardue ML, DeBaryshe PG (2003). Retrotransposons provide an evolutionarily robust non-telomerase mechanism to maintain telomeres. Annual Review of Genetics 37(1): 485-511.
- Parks CG, Miller DB, Mccanlies EC, Cawthon RM, Andrew ME, Deroo LA, Sandler DP (2009). Telomere Length, Current Perceived Stress, and Urinary Stress Hormones in Women. Cancer Epidemiology Biomarkers & Prevention 18: 551-560.
- Partridge L, Alic N, Bjedov I, Piper MDW (2011). Ageing in *Drosophila*: The role of the insulin/Igf and TOR signalling network. Experimental Gerontology 46(5): 376-381.
- Partridge L, Harvey PH. (1985). Cost of reproduction. Nature 316: 20-21.

- Pasupuleti VR, Sammugam L, Ramesh N, Gan SH (2017). Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. Oxidative Medicine and Cellular Longevity 2017: 1259510.
- Patel A, Fondrk MK, Kaftanoglu O, Emore Ch, Hunt G, Frederick K, Amdam GV (2007). The Making of a Queen: TOR Pathway Is a Key Player in Diphenic Caste Development. PLoS ONE 6: e509.
- Pfaffl MW (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic acids research 29(9): e45.
- Pich U, Fuchs J, Schubert I (1996). How do Alliaceae stabilit their chromosome ends in the absence of TTTAGGG sequences? Chromosome research 4(3): 207-213.
- Pich U, Schubert I (1998). Terminal heterochromatin and alternative telomeric sequences in Allium cepa. Chromosome Research 6(4): 315-321.
- Prowse KR, Greider CW (1995). Developmental and tissue-specific regulation of mouse telomerase and telomere length. Proceedings of the National Academy of Sciences of the United States of America 92(11): 4818-4822.
- Raffa GD, Cenci G, Siriaco G, Goldberg ML, Gatti M (2005). The putative Drosophila transcription factor woc is required to prevent telomeric fusions. Molecular Cell 20: 821-831.
- Raffa GD, Ciapponi L, Cenci G, Gatti M (2011). A protein complex that mediates epigenetic maintenance of Drosophila telomeres Terminin. Nucleus 2(5): 383-391.
- Remolina SC, Hughes KA (2008). Evolution and mechanisms of long life and high fertility in queen honey bees. Age 30(2-3): 177-185.
- Reznick D (1985). Costs of Reproduction: An Evaluation of the Empirical Evidence. Oikos 44 (2): 257-267.
- Riha K, Fajkus J, Siroky J, Vyskot B (1998). Developmental Control of Telomere Lengths and Telomerase Activity in Plants. The Plant Cell 10: 1691-1698.

- Romano GH, Harari Y, Yehuda T, Podhorzer A, Rubinstein L, Shamir R, Gottlieb A, Silberberg Y, Pe'er D, Ruppin E, Sharan R, Kupiec M (2013). Environmental Stresses Disrupt Telomere Length Homeostasis. PLoS Genet 9(9): e1003721.
- Rose MR, Finch CE (1994). Genetics and Evolution of Aging. Genetics and Evolution of Aging: p. 5.
- Sahara K, Marec F, Traut W (1999). TTAGG telomeric repeats in chromosomes of some insects and other Arthropods. Chromosome Research 7(6):449-460.
- Salazar-Olivo LA, Paz-González V (2005). Screening of biological activities present in honeybee (Apis mellifera) royal jelly. Toxicology In Vitro 19(5): 645-651.
- Saretzki G (2016). Does telomerase protein protect our neurons? Journal of Neurology Neuromed 1(2): 23-28.
- Sasaki T, Fujiwara H (2000). Detection and distribution patterns of telomerase aktivity in insects. European Journal of Biochemistry 267: 3025-3031.
- Seehuus A-Ch, Norberg N, Gimsa U, Krekling T, Amdam GV (2006). Reproductive protein protects functionally sterile honey bee workers from oxidative stress. PNAS 103 (4): 962-967.
- Seluanov A, Chen ZX, Hine C, Sasahara THC, Ribeiro A, Catania KC, Presgraves DC, Gorbunova V (2007). Telomerase activity coevolves with body mass not lifespan. Aging Cell 6: 45–52.
- Shay JW, Bacchetti S (1997). A survey of telomerase activity in human cancer. European Journal of Cancer 33(5): 787-791.
- Shpiz S, Kwon D, Uneva A, Kim M, Klenov M, Rozovsky Y, Georgiev P, Savitsky M, Kalmykova A (2007). Characterization of Drosophila telomeric retroelement *TAHRE*: transcription, transpositions, and RNAi-based regulation of expression. Molecular Biology and Evolution 24: 2535-2545.

- Scharlaken B, de Graaf DC, Goossens K, Brunain M, Peelman LJ, Jacobs FJ (2008). Reference gene selection for insect expression studies using quantitative real-time PCR: The head of the honey bee, *Apis mellifera*, after bacterial challenge. Journal of Insect Science 8: 33.

- Schubert I, Rieger R, Fuchs J, Pich U (1994). Sequence organization and the mechanism of interstitial deletion clustering in plant genome (*Vicia faba*). Mutation Research 325(1): 1-5.

- Sies H (1997). Oxidative stress: oxidants and antioxidants. Experimental Physiology 82(2): 291-295.

- Smogorzewska A, de Lange T (2004). Regulation of telomerase by telomeric proteins. Annual Review of Biochemistry 73: 177-208.

- Snustad DP, Simmons JM (2000). Principle of Genetics (Second Edition). Learning and Teaching Support Network.

- Suji G, Sivakami S (2004). Glucose, glycation and aging. Biogerontology 5: 365-373.

- Traut W, Szczepanowski M, Vítková M, Opitz C, Marec F, Zrzavý J (2007). The telomere repeat motif of basal Metazoa. Chromosome Research 15(3):371-82.

- Traverse KL, Pardue ML (1988). A Spontaneously Opened Ring Chromosome of *Drosophila Melanogaster* Has Acquired He-T Dna-Sequences at Both New Telomeres. Proceedings of the National Academy of Sciences USA 85(21): 8116-8120.

- Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, Rosenblatt RL, Shay JW, Garcia CK (2007). Adult-onset pulmonary fibrosis caused by mutations in telomerase. PNAS 104: 7552-7557.

- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007). Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology: 39(1): 44-84.

- vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R, Tarpy DR, Pettis JS (2009). Colony collapse disorder: a descriptive study. PloS One 4(8): e6481.

- vanEngelsdorp D, Meixner MD (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology 103: S80-S95.

- Viña J, Borra C, Miquel J (2007). Theories of Ageing. IUBMB Life 59(4-5): 249-254.

- Vítková M, Král J, Traut W, Zrzavý J, Marec F (2005). The evolutionary origin of insect telomeric repeats, (TTAGG)n. Chromosome Research 13: 145-156.

- Viturri R, Colomba MS, Pirrone A, Libertini A (2000). Physical mapping of rDNA genes, (TTAGGG)n telomeric sequence and other karyological features in two earthworms of the family Lumbricidae Annelida: Oligochaeta). Heredity 85 (3): 203-207.

- von Zglinicki T (2002). Oxidative stress shortens telomeres. Trends in Biochemical Sciences 27: 339-344.

- Walter MF, Biessmann H (2004). Expression of the telomeric retrotransposon *HeT-A* in *Drosophila melanogaster* is correlated with cell proliferation. Development Genes and Evolution 214: 211-219.

- Wang Y, Azevedo SV, Hartfelder K, Amdam GV (2013). Insulin-like peptides AmILP1 and AmILP2) differentially affect female caste development in the honey bee (*Apis mellifera* L.). The Journal of Experimental Biology 216, 4347-4357.

- Wei Z, Hurtt R, Gu T, Bodzin AS, Koch WJ, Doria C (2013). GRK2 negatively regulates IGF-1R signaling pathway and cyclins' expression in HepG2 cells. Journal of Cell Physiology 228(9): 1897-1901.

- Williamson JR, Raghuraman MK, Cech TR (1989). Monovalent cation-induced structure of telomeric DNA: the G-quartet model. Cell 59(5): 871-880.

- Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW (1996). Telomerase Activity in Human Germline and Embryonic Tissues and Cells. Developmental Genetics 18: 173-179.
- Wyatt HDM, West SC, Beattie TL (2010). InTERTpreting telomerase structure and fiction. Nucleic Acids Research 38(17): 5609-5622.
- Yasumoto S, Kunimura C, Kikuchj K, Tahara H, Ohji H, Yamamoto H, Ide T, Utakoji T (1996). Telomerase activity in normal human epithelial cells. Oncogene 13(2): 433-439.
- Yasunari K, Maeda K, Nakamura M, Yoshikawa J (2002). Pressure promotes angiotensin II-mediated migration of human coronary smooth muscle cells through increase in oxidative stress. Hypertension 39(2): 433-437.
- Yui J, Chiu CP, Lansdorp PM (1998). Telomerase activity in candidate stem cells from foetal liver and adult bone marrow. Blood 91: 3255-3262.
- Zakian VA (1995). Telomeres: Beginning to understand the end. Science 270: 1601-1607.
- Zarse K, Schmeisser S, Groth M, Priebe S, Beuster G, Kuhlow D, Guthke R, Platzer M, Kahn CR, Ristow M (2012). Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metabolism 15(4): 451-465.
- Zemanová M, Stašková T, Kodrík D (2016). Role of adipokinetic hormone and adenosine in the anti-stress response in *Drosophila melanogaster*. Journal of Insect Physiology 91-92: 39-47.
- Zhou J, Ding D, Wang M, Cong YS (2014). Telomerase reverse transcriptase in the regulation of gene expression. BMB Reports 47(1): 8-14.

7. APPENDIX

I. Sequences of used primers

I.I. Sequences of primers for *A. mellifera*

Gene	primer	sequence
Juvenile hormon	Juv1F	5´- AATTGTTGGACTCAAACAGAAA - 3´
	Juv1R	5´- TAAAATTAGCGAGAGTTTCAAC - 3´
Target of Rapamycin	Tor1F	5´- GATTACACGTGTCTGCCTC - 3´
	Tor1R	5´- CTTAGTGCTGGTGATGGTG - 3´
Insulin-like Receptor	InRF1	5´- CCGGAGCTGAGAGAGATC - 3´
	InRR1	5´- ATCTCGAACGCGACGAGG - 3´
Vittelogenin	Vit1F	5´- ATGGTCGACAATCCAGAATC - 3´
	Vit1R	5´- GCTTCAACTTTTCTTCGCTC - 3´
RpL13A	RpL13amellifera A	5´- TGGCCATTTACTGGTCGTT - 3´
	RpL13amellifera B	5´- GAGCACGGAAATGAAATGGT - 3´
Actin	Act mellifera A	5´- TGCCAACACTGTCCTTTCTG - 3´
	Act mellifera B	5´- AGAATTGACCCACCAATCCA - 3´

I.II. Sequence of primers for *D. melanogaster*

Gene	Primer	Sequence
RpL 32	RpL 32 F	5´– ATTGTCTTCTCCTCCGTCCACC – 3´
	RpL 32 R	5´– TTCTCTATGCTATTGTCGCTGTGC – 3´
Actin 42A	Act 79PO F	5´ – TGTATGCCAACAATGTGC – 3´
	Act 79PO R	5´– ACAACGAAGCCAGGATGGAG – 3´
HeT-A	HeT-A F	5´– ATTGTCTTCTCCTCCGTCCACC – 3´
	HeT-A R	5´– TTCTCTATGCTATTGTCGCTGTGC – 3´
White	WExo F	5´– CCTCTTTATCGGCTCCCTAACG – 3´
	WExo R	5´– TCGTGTGCTGACATTTGCTGAG – 3´
Gypsy	Gypsy F	5´– GCTAGAGAAGCAGAGGCAAG – 3´
	Gypsy R	5´– GTCCTTGTTGGTTTGTCCGT – 3´
Jockey	Jockey F	5´– ATATGACCGGGTTCGCTTTG – 3´
	Jockey R	5´– GTTGTGGAGTTTGAGTGTG – 3´
I-element	I-el F	5´– GGGATGCAAACGTACAAGAG – 3´
	I-el R	5´– GTCGATGTGTCTAAGGTCGT – 3´

II. Results from Bonferroni´s multiple comparison test

Bonferroni's Multiple Comparison Test	P value
Nov-Dec (2014) vs Jan-Feb (2015)	P > 0.05
Nov-Dec (2014) vs March-April (2015)	P > 0.05
Nov-Dec (2014) vs May-June (2015)	P < 0.01
Nov-Dec (2014) vs July-Aug (2015)	P < 0.01
Nov-Dec (2014) vs Sept-Oct (2015)	P < 0.05
Nov-Dec (2014) vs Nov-Dec (2015)	P > 0.05
Nov-Dec (2014) vs Jan-Feb (2016)	P > 0.05
Nov-Dec (2014) vs March-April (2016)	P > 0.05
Jan-Feb (2015) vs March-April (2015)	P > 0.05
Jan-Feb (2015) vs May-June (2015)	P > 0.05
Jan-Feb (2015) vs July-Aug (2015)	P > 0.05
Jan-Feb (2015) vs Sept-Oct (2015)	P > 0.05
Jan-Feb (2015) vs Nov-Dec (2015)	P > 0.05
Jan-Feb (2015) vs Jan-Feb (2016)	P > 0.05
Jan-Feb (2015) vs March-April (2016)	P > 0.05
March-April (2015) vs May-June (2015)	P > 0.05
March-April (2015) vs July-Aug (2015)	P > 0.05
March-April (2015) vs Sept-Oct (2015)	P > 0.05
March-April (2015) vs Nov-Dec (2015)	P > 0.05
March-April (2015) vs Jan-Feb (2016)	P < 0.05
March-April (2015) vs March-April (2016)	P > 0.05
May-June (2015) vs July-Aug (2015)	P > 0.05
May-June (2015) vs Sept-Oct (2015)	P > 0.05
May-June (2015) vs Nov-Dec (2015)	P > 0.05
May-June (2015) vs Jan-Feb (2016)	P < 0.001
May-June (2015) vs March-April (2016)	P > 0.05
July-Aug (2015) vs Sept-Oct (2015)	P > 0.05
July-Aug (2015) vs Nov-Dec (2015)	P < 0.05
July-Aug (2015) vs Jan-Feb (2016)	P < 0.001
July-Aug (2015) vs March-April (2016)	P > 0.05
Sept-Oct (2015) vs Nov-Dec (2015)	P > 0.05
Sept-Oct (2015) vs Jan-Feb (2016)	P < 0.01
Sept-Oct (2015) vs March-April (2016)	P > 0.05
Nov-Dec (2015) vs Jan-Feb (2016)	P > 0.05
Nov-Dec (2015) vs March-April (2016)	P > 0.05
Jan-Feb (2016) vs March-April (2016)	P > 0.05

Statistically significant differences are marked in red colour.

III. Supplemented data to the manuscript

S1.PCR primers used in this study.

Primer	Sequence (5'-3')
w1F	5’-TTGGAAAACTCGGATCTTGG-3’
w1R	5’-CGTACCTCTCATGGTTCCGT-3’
w2F	5’-CGAGCTTCACTCAACCAACA-3’
w2R	5’-TGAATAATTGCGCCTCCTTC-3’
HeT-A (forward)	5’-ATTGTCTTCTCCTCCGTCCACC-3’
HeT-A (reverse)	5’-TTCTCTATGCTATTGTCGCTGTGC-3’
Tart (forward)	5’-TAGAACGGACGAGGACAAAG-3’
Tart (reverse)	5’-CCTTCATCTAGCAGTCTCCA-3’
TAHRE (forward)	5’-TCAAAGGCTTCCACACCTAC-3’
TAHRE (reverse)	5’-AGGGGATAAGTGCGATGGGT-3’
Jockey (forward)	5’-ATATGACCGGGTTCGCTTTG-3’
Jockey (reverse)	5’-GTTGTGGAGGTTTGAGTGTG-3’
RpL32 (forward)	5’- GGACAGTATCTGATGCCCAAC-3’
RpL32 (reverse)	5’- ATCTCGCCGCAGTAAACGC-3’
white (forward)	5’- CCTCTTTATCGGCTCCCTAACG-3’
white (reverse)	5’- TCGTGTGCTGACATTTGCTGAG-3’
actin 42A (forward)	5’-AAGAGGTTGCAGCTTTAGTGG-3’
actin 42A (reverse)	5’- GCCGACATAAGAGTCCTTTTG - 3’

S2. Statistical analysis of *HeT-A* copy numbers in generation 1

	Paraquat concentration						
	0 nM	10^{-1} mM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	92,358	98,4698	97,6041	120,6866	106,7299	106,8459	110,9534
	89,08689	90,68517	92,9121	105,62	112,1741	92,59065	95,19373
	72,39632	85,7934	76,25683	103,0924	76,78724	98,55073	63,02248
	93,8954	112,0239	76,1849	101,9417	113,6495	101,4602	89,43668
	88,47466	99,7285	116,7918	107,0985	114,3882	78,94602	64,34672
	75,64453		84,61225	103,2318	111,2867	85,1839	110,9534

One-way analysis of variance	
P value	0,0655
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	7
F	2,138
R squared	0,2075

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	30,53
P value	P<0.0001
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	5083	6	847,2
Residual (within columns)	19410	49	396,2
Total	24500	55	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	4,193	0,4213	P > 0.05	-22.30 to 30.69
0 mM vs 10-2 mM	-5,418	0,5444	P > 0.05	-31.91 to 21.08
0 mM vs 10-3 mM	-21,64	2,174	P > 0.05	-48.13 to 4.857
0 mM vs 10-4 mM	-20,53	2,063	P > 0.05	-47.02 to 5.966
0 mM vs 10-5 mM	-8,620	0,8662	P > 0.05	-35.11 to 17.87
0 mM vs 10-6 mM	0,7187	0,07222	P > 0.05	-25.77 to 27.21

S3. Statistical analysis of *HeT-A* copy numbers in generation 2

	Paraquat concentration						
	0 nM	10^{-1} mM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	80,46874	84,02779	72,64516	90,05875	72,39382	102,3803	65,92686
	91,45894	75,20698	107,0985	91,31593	106,3587	102,0261	101,3214
	100,2454	90,05875	105,6241	104,5315	83,15866	97,86999	109,3489
	93,98306	78,21699	101,6731	137,453	150,9362	100,9708	106,3587
	84,69875	89,04475	73,15044	73,15044	101,3214	79,77112	84,02779
	91,74247	80,05175	92,27031	108,8021	99,23621	79,2201	80,88468
	82,43648	72,24599	81,44728			52,44723	49,6181

One-way analysis of variance	
P value	0,2201
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	7
F	1,436
R squared	0,1496

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	18,68
P value	0,0047
P value summary	**
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	2911	6	485,1
Residual (within columns)	16550	49	337,8
Total	19460	55	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
Column A vs 10-1 mM	8,026	0,8734	P > 0.05	-16.44 to 32.49
Column A vs 10-2 mM	-1,268	0,1380	P > 0.05	-25.73 to 23.19
Column A vs 10-3 mM	-11,59	1,262	P > 0.05	-36.06 to 12.87
Column A vs 10-4 mM	-12,94	1,409	P > 0.05	-37.40 to 11.52
Column A vs 10-5 mM	1,478	0,1609	P > 0.05	-22.98 to 25.94
Column A vs 10-6 mM	3,935	0,4283	P > 0.05	-20.53 to 28.40

S4. Statistical analysis of *HeT-A* copy numbers in generation 3

	Paraquat concentration						
	0 nM	10^{-1} mM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	84,3568	110,8753	64,57011	103,4503	112,4231	65,01923	55,63013
	85,28328	85,49657	108,2178	134,1585	117,1972	85,20078	90,37141
	105,0642	86,69006	113,5981	120,4921	144,2867	112,4231	125,6089
	90,63444	65,24496	111,2603	180,7428	110,7428	107,8434	128,2482
	88,86426	115,985	89,43668	110,8753	110,8753	55,24587	76,52158
	94,36749	111,6465	118,4221	125,1743	125,1743	83,73708	80,04807
	84,3568	110,8753	64,57011	103,4503	112,4231	65,01923	55,63013

One-way analysis of variance	
P value	0,0009
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	7
F	4,598
R squared	0,3602

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	12,84
P value	0,0456
P value summary	*
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	12470	6	2078
Residual (within columns)	22150	49	451,9
Total	34610	55	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-8,121	0,7640	P > 0.05	-36.42 to 20.17
0 mM vs 10-2 mM	-9,489	0,8927	P > 0.05	-37.78 to 18.81
0 mM vs 10-3 mM	-37,72	3,549	P < 0.01	-66.02 to -9.425
0 mM vs 10-4 mM	-28,69	2,699	P < 0.05	-56.98 to -0.3924
0 mM vs 10-5 mM	6,517	0,6131	P > 0.05	-21.78 to 34.81
0 mM vs 10-6 mM	-1,310	0,1232	P > 0.05	-29.61 to 26.99

S5. Statistical analysis of *HeT-A* copy numbers in generation 4

	Paraquat concentration						
	0 nM	10^{-1} mM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	91,13921	122,1741	97,53139	136,5036	138,8896	123,8796	132,7709
	87,1241	110,4917	118,0124	132,7709	118,8332	124,7412	112,4231
	89,44851	98,20977	102,0261	82,87096	120,2603	105,6241	91,63296
	93,59323	106,3587	99,58073	146,8088	115,5837	110,4917	124,7412
	95,65385	112,4231	67,31213	136,0313	122,5983	65,92686	79,77112
	92,98754	95,19373	117,6041	117,1972	126,4826	104,8945	80,32597
	87,75674	67,78033	97,53139	88,81889	127,3623	66,84718	87,59609
	91,13921	122,1741		136,5036	138,8896	123,8796	132,7709

One-way analysis of variance	
P value	0,0082
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	7
F	3,310
R squared	0,2884

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	26,42
P value	0,0002
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	6871	6	1145
Residual (within columns)	16950	49	346,0
Total	23820	55	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-10,70	1,151	P > 0.05	-35.46 to 14.05
0 mM vs 10-2 mM	-9,244	0,9940	P > 0.05	-34.00 to 15.51
0 mM vs 10-3 mM	-29,04	3,123	P < 0.05	-53.80 to -4.286
0 mM vs 10-4 mM	-33,19	3,568	P < 0.01	-57.94 to -8.430
0 mM vs 10-5 mM	-9,243	0,9939	P > 0.05	-34.00 to 15.51
0 mM vs 10-6 mM	-10,22	1,099	P > 0.05	-34.98 to 14.53

S6. Statistical analysis of *HeT-A* copy numbers in generation 5

	Paraquat concentration						
	0 nM	10^{-1} mM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	90,86325	105,9908	88,5116	128,6934	173,982	125,1743	104,5315
	104,9363	145,7947	145,2903	172,1825	166,8947	155,7183	169,2245
	97,42974	105,2586	142,3003	112,4231	166,3173	135,5607	130,4899
	82,64679	108,5936	115,985	121,3302	137,9302	120,4921	60,8757
	90,65634	138,8896	122,5983	104,5315	144,2867	115,207	93,23467
	94,65333	98,20977	105,6241	141,808	157,3458	127,8929	92,9121
	89,98763	105,9908	119,6598	118,0124	158,9903	101,3972	97,86999
	90,86325		88,5116	128,6934	173,982	125,1743	104,5315

One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	7
F	7,641
R squared	0,4834

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	16,25
P value	0,0125
P value summary	*
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	19510	6	3252
Residual (within columns)	20850	49	425,6
Total	40360	55	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-24,10	2,336	P > 0.05	-51.56 to 3.359
0 mM vs 10-2 mM	-26,97	2,615	P > 0.05	-54.43 to 0.4864
0 mM vs 10-3 mM	-35,40	3,432	P < 0.01	-62.86 to -7.944
0 mM vs 10-4 mM	-64,94	6,296	P < 0.01	-92.40 to -37.48
0 mM vs 10-5 mM	-32,90	3,189	P < 0.05	-60.35 to -5.438
0 mM vs 10-6 mM	-14,00	1,357	P > 0.05	-41.45 to 13.46

S7. Statistical analysis of *TART* copy numbers in generation 1

	Paraquat concentration				
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
TART copy number	112,49	97,99	61,98	111,68	35,21
	103,42	137,45	131,42	101,84	108,35
	81,56	75,8	89,58	80,6	100,2
	105,43	68,3302	103,42	97,89	91,59
	93,354	103,42	103,42	101,29	87,19

One-way analysis of variance	
P value	0,8170
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	5
F	0,3846
R squared	0,07142

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	5,139
P value	0,2733
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

ANOVA Table	SS	df	MS
Treatment (between columns)	760,5	4	190,1
Residual (within columns)	9887	20	494,4
Total	10650	24	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	2,653	0,1886	P > 0.05	-34.61 to 39.92
0 mM vs 10-3 mM	1,287	0,09151	P > 0.05	-35.98 to 38.55
0 mM vs 10-4 mM	0,5908	0,04201	P > 0.05	-36.67 to 37.86
0 mM vs 10-6 mM	14,74	1,048	P > 0.05	-22.52 to 52.01

S8. Statistical analysis of *TART* copy numbers in generation 5

	Paraquat concentration				
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
TART copy number	89,73	86,36	97,51	100,61	93,89
	106,46	104,11	121,56	119,93	97,79
	87,89	142,6	145,03	129,34	102,63
	101,1203	101,92	103,42	137,79	132,63
	75,258	103,42	103,42	138,19	101,43

One-way analysis of variance	
P value	0,0719
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	5
F	2,540
R squared	0,3368

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	1,257
P value	0,8686
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

ANOVA Table	SS	df	MS
Treatment (between columns)	2936	4	733,9
Residual (within columns)	5780	20	289,0
Total	8716	24	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-15,59	1,450	P > 0.05	-44.08 to 12.90
0 mM vs 10-3 mM	-22,10	2,055	P > 0.05	-50.59 to 6.396
0 mM vs 10-4 mM	-33,08	3,077	P < 0.05	-61.57 to -4.588
0 mM vs 10-6 mM	-13,58	1,263	P > 0.05	-42.07 to 14.91

S9. Statistical analysis of *TAHRE* copy numbers in generation 1

	Paraquat concentration				
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
TAHRE copy number	98,49	154,3	92,98	99,26	71,9
	112,08	88,35	95,7	81,31	102,14
	90,89	75,8	88,96	81,73	111,18
	98,02	81,35	97,7	71,41	103,34
	91,18	77,9	88,96	69,78	115,28

One-way analysis of variance	
P value	0,4149
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	5
F	1,032
R squared	0,1711

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	14,32
P value	0,0064
P value summary	**
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	1372	4	342,9
Residual (within columns)	6643	20	332,2
Total	8015	24	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	5,392	0,4678	P > 0.05	-25.15 to 35.94
0 mM vs 10-3 mM	8,072	0,7003	P > 0.05	-22.47 to 38.62
0 mM vs 10-4 mM	20,23	1,755	P > 0.05	-10.31 to 50.78
0 mM vs 10-6 mM	0,1640	0,01423	P > 0.05	-30.38 to 30.71

S10. Statistical analysis of *TAHRE* copy numbers in generation 5

	Paraquat concentration				
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
TAHRE copy number	89,73	86,36	97,51	115,61	93,89
	106,46	104,11	121,56	119,93	97,79
	87,89	142,6	145,03	129,34	102,63
	101,1203	101,92	103,42	137,79	132,63
	75,258	103,42	105,58	138,19	101,43

One-way analysis of variance	
P value	0,0318
P value summary	*
Are means signif. different? (P < 0.05)	Yes
Number of groups	5
F	3,287
R squared	0,3966

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	12,29
P value	0,0153
P value summary	*
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	8720	4	2180
Residual (within columns)	13270	20	663,3
Total	21990	24	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-19,21	1,179	P > 0.05	-62.37 to 23.96
0 mM vs 10-3 mM	-38,94	2,390	P > 0.05	-82.10 to 4.227
0 mM vs 10-4 mM	-54,76	3,362	P < 0.05	-97.93 to -11.60
0 mM vs 10-6 mM	-20,56	1,262	P > 0.05	-63.73 to 22.60

S11. Statistical analysis of *HeT-A* transcript in Oregon R

Generation 1:

	Paraquat concentration					
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	0,003488	0,014665	0,009484	0,001925	0,024598	**0,016323**
	0,005671	0,00326	0,003594	0,012632	0,031864	**0,015897**
	0,0049	0,0198	0,012296	0,00566	0,007835	**0,017385**
	0,009645	0,005906	0,03117	0,0257	0,022902	**0,023337**
	0,003488	0,014665	0,009484	0,001925	0,024598	**0,016323**

One-way analysis of variance	
P value	0,1775
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	7
P value	1,672
P value summary	0,3232

ANOVA Table	SS	df	MS
Treatment (between columns)	0,001032	6	0,0001721
Residual (within columns)	0,0003687	21	0,00001756
Total	0,001401	27	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-0,004982	0,8573	P > 0.05	-0.02119 to 0.01123
0 mM vs 10-2 mM	-0,008210	1,413	P > 0.05	-0.02442 to 0.008002
0 mM vs 10-3 mM	-0,005553	0,9557	P > 0.05	-0.02177 to 0.01066
0 mM vs 10-4 mM	-0,01587	2,732	P > 0.05	-0.03209 to 0.0003384
0 mM vs 10-5 mM	-0,01231	2,118	P > 0.05	-0.02852 to 0.003903

Generation 5:

	Paraquat concentration					
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	0,005279	0,013669	0,009344	0,019246	0,024598	0,015323
	0,005668	0,01325	0,006584	0,019632	0,021864	0,012887
	0,005919	0,009801	0,010296	0,02566	0,026825	0,016375
	0,008649	0,006906	0,021172	0,0247	0,022902	0,022338
	0,005279	0,013669	0,009344	0,019246	0,024598	0,015323

One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	7
P value	P<0.0001
P value summary	***

Bartlett's test for equal variances	
Treatment (between columns)	0,001032
Residual (within columns)	0,0003687
Total	0,001401
Treatment (between columns)	0,001032

ANOVA Table	SS	df	MS
Treatment (between columns)	0,001032	6	0,0001721
Residual (within columns)	0,0003687	21	0,00001756
Total	0,001401	27	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-0,004528	1,528	P > 0.05	-0.01279 to 0.003738
0 mM vs 10-2 mM	-0,005471	1,847	P > 0.05	-0.01374 to 0.002795
0 mM vs 10-3 mM	-0,01593	5,377	P < 0.01	-0.02420 to -0.007665
0 mM vs 10-4 mM	-0,01767	5,964	P < 0.01	-0.02593 to -0.009403
0 mM vs 10-5 mM	-0,01035	3,494	P < 0.05	-0.01862 to -0.002087

S12. Statistical analysis of *HeT-A* transcript in $y^1 w^{67c2}$; Hetom line

	Paraquat concentration					
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeT-A copy number	0,000108	0,000228	0,000495	0,000833	0,000354	0,000349
	0,000145	0,00015	0,000323	0,000413	0,000143	0,000522
	0,000154	0,000126	0,0001	0,000512	0,00021	0,00015
	0,000254	0,00027	0,00058	0,00061	0,00028	0,000251
	0,000108	0,000228	0,000495	0,000833	0,000354	0,000349

One-way analysis of variance	
P value	0,0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	8,403
R squared	0,6365

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	10,35
P value	0,0659
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

ANOVA Table	SS	df	MS
Treatment (between columns)	0,000005037	5	0,000001007
Residual (within columns)	0,000002877	24	0,0000001199
Total	0,000007914	29	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-0,0003898	1,780	P > 0.05	-0.0009811 to 0.0002014
0 mM vs 10-3 mM	-0,001087	4,963	P < 0.01	-0.001678 to -0.0004956
0 mM vs 10-4 mM	-0,0008984	4,103	P < 0.01	-0.001490 to -0.0003072
0 mM vs 10-5 mM	-0,00002264	0,1034	P > 0.05	-0.0006139 to 0.0005686
0 mM vs 10-6 mM	-0,0003394	1,550	P > 0.05	-0.0009307 to 0.0002518

S13. Statistical analysis of *HeTom* transcript levels in $y^1 w^{67c23}$; *HeTom* line

	Paraquat concentration					
	0 nM	10^{-2} mM	10^{-3} mM	10^{-4} mM	10^{-5} mM	10^{-6} mM
HeTom transcript levels	0,000818	0,000916	0,001353	0,00151	0,000802	0,000927
	0,000596	0,000779	0,00192	0,001609	0,001114	0,001029
	0,000798	0,0015	0,0015	0,0019	0,0004	0,00153
	0,000912	0,00149	0,0027	0,0017	0,0009	0,000997
	0,000818	0,000916	0,001353	0,00151	0,000802	0,000927

One-way analysis of variance	
P value	0,0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	8,403
R squared	0,6365

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	10,35
P value	0,0659
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

ANOVA Table	SS	df	MS
Treatment (between columns)	0,000005037	5	0,000001007
Residual (within columns)	0,000002877	24	0,0000001199
Total	0,000007914	29	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10-1 mM	-0,0003898	1,780	P > 0.05	-0.0009811 to 0.0002014
0 mM vs 10-3 mM	-0,001087	4,963	P < 0.01	-0.001678 to -0.0004956
0 mM vs 10-4 mM	-0,0008984	4,103	P < 0.01	-0.001490 to -0.0003072
0 mM vs 10-5 mM	-0,00002264	0,1034	P > 0.05	-0.0006139 to 0.0005686
0 mM vs 10-6 mM	-0,0003394	1,550	P > 0.05	-0.0009307 to 0.0002518

S14. Statistical analysis of HeTom fluorescence

Paraquat concentration	Fluorescence				
0 mM	272349,0	194344,0	305554,0	395996,0	478834,5
	98651,0	292818,0	254331,0	220560,0	320183,5
	527772,0	253747,0	239253,0	218124,0	242648,5
	239449,0	384985,0	442122,0	318975,0	509994,0
	366894,0	496023,0	306417,0	197918,0	462947,5
	281319,0	420105,0	535577,0	149510,0	336236,0
	235119,0	345586,0	278392,0	157612,0	386759,5
	123899,0	333483,0	348107,0	339185,0	540208,0
	301611,0	371306,0	321279,0	311215,0	540549,0
	232899,0	414545,0	666309,0	184860,0	439318,5
	143644,0	239276,0	324879,0	153140,0	337422,0
	348061,0	315396,0	457528,0	246673,0	328154,5
	207243,0	206608,0	303101,0	243585,0	174597,0
	246377,0	421125,0	271739,0	390369,0	454602,0
	277945,0	286515,0	198996,0	288927,0	347223,5
	241651,0	372507,0	145483,0	195021,0	259664,0
	288469,0	282818,0	134052,0	222853,0	197346,0
	298132,0	353541,0	262773,0	259679,0	484209,0
	287828,0	283163,0	186414,0	326956,0	432466,0
	282945,0	164260,0	165316,0	315955,0	154891,5
	138611,0	322366,0	116386,0	165546,0	383249,0
	345129,0	387617,0	299226,0	154156,0	722247,5
	232703,0	310134,0	176203,0	213491,0	420461,0
	230932,0	287132,0	223931,0	181001,5	422230,5
	122436,0	418740,0	385702,0	131209,5	630417,5
	322283,0	346032,0	216562,0	203580,5	215347,0
	167588,0	203810,0	315866,0	164928,0	379314,0
	250455,0	283903,0	165464,0	276406,0	329992,5
	320089,0	301218,0	315157,0	248558,0	590551,5
	224454,0	331592,0	335901,0	478834,5	347805,0
	663042,5	584646,5	347512,5	396187,5	398012,8
	456838,0	267735,0	394149,3	212238,0	386502,0
	396187,5	289984,5	212238,0	463263,5	267692,0

Paraquat concentration	Fluorescence				
10^{-4} mM	391183,0	345685,0	467169,0	482041,0	409025,0
	407835,0	222973,0	304265,0	409312,0	494332,0
	441661,0	206160,0	584407,0	587709,0	342764,5
	360440,0	307186,0	370172,0	376135,0	359433,0
	344377,0	551382,0	308460,0	234904,0	288435,5
	405940,0	428373,0	416389,0	314603,0	404520,0
	429839,0	267390,0	546999,0	517955,0	327090,5
	321293,0	323020,0	311044,0	538547,0	327961,5
	582919,0	287421,0	396596,0	314954,0	292270,0
	293502,0	307073,0	196725,0	170313,0	201703,5
	311696,0	392656,0	296395,0	425591,0	396244,5
	351912,0	379335,0	509366,0	410572,0	371086,5
	164160,0	214337,0	248172,0	233566,0	393666,5
	471187,0	471166,0	398741,0	591698,0	447350,0
	402147,0	408762,0	296178,0	307100,0	290389,0
	541117,0	572422,0	379242,0	243085,0	658268,0
	579978,0	400118,0	161347,0	358435,0	325216,0
	307160,0	592176,0	345064,0	378872,0	298608,0
	187858,0	545811,0	379007,0	513645,0	679499,0
	286968,0	221864,0	248159,0	513469,5	248227,0
	243763,0	284884,0	579144,0	264742,0	410395,0
	484057,0	259547,0	340857,0	421865,0	577497,0
	402554,0	250542,0	288014,0	227730,5	488048,5
	341200,0	519881,0	317531,0	207116,5	420852,0
	320755,0	307193,0	496290,0	161252,5	455708,0
	402477,0	320099,0	365928,0	245034,5	195209,0
	273965,0	284997,0	589470,0	566402,0	312530,0
	307293,0	323491,0	192030,0	544899,0	378396,5
	153391,0	269148,0	642850,0	593356,0	672430,0
	411225,0	220647,0	632045,0	409025,0	457680,5
	644731,5	454560,0	411824,5	374878,5	303852,5
	462163,5	374136,5	417014,5	404088,0	546906,0
	185289,5	690063,0	424642,0	286023,5	380374,5
	302787,5	888368,0	340065,5	457589,0	190673,5
	482718,5	313972,5			

Paraquat concentration	Fluorescence				
10^{-6} mM	436224,0	335515,0	278000,0	290288,0	306610,0
	267476,0	330190,0	343766,0	291041,0	461078,0
	224840,0	621818,0	325400,0	471011,0	352196,0
	202707,0	293267,0	408046,0	459423,0	317145,0
	580423,0	377452,0	212797,0	442740,0	200317,5
	332957,0	352850,0	302526,0	283005,0	314773,5
	642903,0	363165,0	239366,0	408075,0	217646,0
	386803,0	285275,0	390263,0	238976,0	392148,5
	627715,0	244294,0	209708,0	571637,0	457278,0
	542230,0	218130,0	340320,0	263514,0	317786,5
	342562,0	341790,0	291116,0	249551,0	198955,0
	304664,0	272909,0	376502,0	159208,0	194887,0
	554257,0	233055,0	194158,0	465815,0	360492,5
	330436,0	373702,0	491273,0	246716,0	458779,5
	480558,0	306320,0	209497,0	311590,5	282175,5
	420214,0	215698,0	358390,0	434443,0	776364,5
	150075,0	194946,0	281040,0	387803,0	227176,0
	201424,0	344827,0	451665,0	441307,5	574187,5
	375397,0	301734,0	223999,0	448246,0	396813,0
	349285,0	260207,0	375277,0	436502,5	496281,0
	532351,0	150396,0	241763,0	256290,5	359505,5
	284508,0	287210,0	322451,0	478190,5	420325,5
	147826,0	341817,0	295715,0	404035,0	380406,0
	172401,0	373760,0	411612,0	266995,0	418855,5
	85194,0	291461,0	242010,0	449856,0	358224,0
	291793,0	458670,0	486699,0	136667,5	325057,5
	280374,0	227385,0	592894,0	396943,5	217268,0
	427229,0	216201,0	360997,0	419228,0	520506,5
	347457,0	333657,0	507032,0	223797,0	384539,5
	377692,0	358500,0	156882,0	334365,5	225446,0
	482273,0	593228,0	298980,0	634523,5	593364,5
	317805,0	382752,0	386014,0	335170,0	655087,5
	239214,0	389998,0	373114,0	425805,0	278993,5
	252457,0	402884,5	275052,0	178021,0	

Paraquat concentration	Fluorescence				
10^{-1} mM	263810,5	282860,0	271084,0	326669,0	542730,0
	204076,0	426811,0	159847,0	220248,0	324465,0
	592936,0	312108,0	305243,0	239727,0	680052,0
	150179,5	223630,5	278045,0	390256,0	365381,0
	489083,5	433207,0	236837,0	279561,0	422178,0
	562555,5	476003,5	289467,0	307540,0	351669,0
	393867,5	638268,5	454435,0	313089,0	483103,0
	384702,0	495084,0	269500,0	536211,0	286448,0
	474979,0	534024,0	239991,0	262343,0	299254,0
	414298,5	500453,0	355883,0	458810,0	454858,0
	207565,5	470514,5	209173,0	307959,0	316022,0
	319921,0	434128,5	224024,0	337400,0	368050,0
	303738,5	178878,0	251904,0	270242,0	400654,0
	305906,0	582553,5	181294,0	317056,0	254143,0
	382476,0	434650,0	327159,0	446442,0	135908,0
	402078,0	443805,5	290368,0	419094,0	455145,0
	319056,5	570972,0	348123,0	226283,0	186393,0
	672286,0	562767,0	256882,0	532437,0	93818,0
	440921,5	384834,0	378161,0	477926,0	119625,0
	427559,0	372241,0	160425,0	244626,0	452944,0
	440059,5	417667,5	333863,0	253961,0	228090,0
	444977,5	313455,5	284660,0	428733,0	436871,0
	322427,0	470462,5	398211,0	561702,0	250875,0
	449591,0	620540,0	263385,0	540374,0	245821,0
	641434,0	118178,0	477918,0	173802,0	161466,0
	586258,0	146991,0	307262,0	334147,0	243834,0
	463921,5	343673,0	357150,0	246376,0	126234,0
	325823,0	251998,0	441034,0	542730,0	376352,0
	237420,0	190916,0	313298,0	222829,0	346446,0
	333323,0	116735,0	378684,0	373182,0	560748,0
	380788,0	122478,0	372610,0	465338,0	384402,0
	261982,0	191516,0	352234,0	235138,0	244202,0
	124700,0	271084,0	369827,0	191317,0	188882,0
	118606,0	198423,0	171718,0	235138,0	428104,0
	250279,0	401424,0	465338,0	191317,0	328759,0
	176419,0	381301,0	235138,0	235138,0	171718,0
	193406,0	410824,0	191317,0	191317,0	465338,0

One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
F	10,06
R squared	0,04297

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	2,131
P value	0,5456
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

ANOVA Table	SS	df	MS
Treatment (between columns)	470000000000	3	156700000000
Residual (within columns)	10470000000000	672	15580000000
Total	10940000000000	675	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10^{-3} mM	56,77	4,856	P < 0.01	26.32 to 87.23
0 mM vs 10^{-3} mM	56,77	4,856	P < 0.01	26.32 to 87.23
0 mM vs 10^{-3} mM	56,77	4,856	P < 0.01	26.32 to 87.23

S15. Non-telomeric retroelement *Jockey*

Transcript level of non-telomeric retroelement *Jockey* in the 1st generation

	Paraquat concentration				
	0 mM	10^{-1} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
Transcript level	0,364755	0,329877	0,473029	0,641713	1,035077
	0,784584	0,765779	1,231144	1,235419	2,12874
	0,469761	0,38289	0,568409	1,01396	0,114036
	0,072154	0,54123	1,32121	0,98741	0,82547

One-way analysis of variance	
P value	0,2650
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	5
F	1,454
R squared	0,2795

ANOVA Table	SS	df	MS
Treatment (between columns)	SS	df	MS
Residual (within columns)	1,250	4	0,3124
Total	3,222	15	0,2148

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10^{-1} mM	-0,08213	0,2506	P > 0.05	-0.9768 to 0.8126
0 mM vs 10^{-3} mM	-0,4756	1,451	P > 0.05	-1.370 to 0.4191
0 mM vs 10^{-4} mM	-0,5468	1,669	P > 0.05	-1.441 to 0.3479
0 mM vs 10^{-6} mM	-0,6030	1,840	P > 0.05	-1.498 to 0.2917

Transcript level of non-telomeric retroelement *Jockey* in the 5th generation

One-way analysis of variance	
P value	0,0009
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	5
F	8,356
R squared	0,6902

ANOVA Table	SS	df	MS
Treatment (between columns)	5,251	4	1,313
Residual (within columns)	2,357	15	0,1571
Total	7,608	19	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10^{-1} mM	-0,3693	1,317	P > 0.05	-1.134 to 0.3959
0 mM vs 10^{-3} mM	-1,089	3,886	P < 0.01	-1.854 to -0.3240
0 mM vs 10^{-4} mM	-0,8032	2,866	P < 0.05	-1.568 to -0.03801
0 mM vs 10^{-6} mM	-1,448	5,168	P < 0.01	-2.214 to -0.6832

	Paraquat concentration				
	0 mM	10^{-1} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
Transcript level	0,4003266	0,9843388	1,630104	1,200621	2,065846
	0,4297998	0,478711	1,140735	0,9684713	2,198825
	0,2841138	0,8964077	2,136077	1,679995	0,953783
	0,4641981	0,6960326	1,028088	0,9419884	2,153574

S16. Statistical analysis of transcript level of *HeT-A* element after H_2O_2 treatment

	H_2O concentration			
	0 %	10^{-3} %	10^{-4} %	10^{-6} %
Transcript level	0,002472	0,006968	0,004597	0,001906
	0,002677	0,004826	0,002705	0,001118
	0,003401	0,00423	0,002687	0,000914
	0,003687	0,005282	0,003187	0,001218

One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
F	16,05
R squared	0,7506

ANOVA Table	SS	df	MS
Treatment (between columns)	0,00003509	3	0,00001170
Residual (within columns)	0,00001166	16	0,0000007288
Total	0,00004675	19	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 % vs 10-3 %	-0,002403	4,450	P < 0.01	-0.003801 to -0.001004
0 % vs 10-4 %	-0,0001896	0,3511	P > 0.05	-0.001588 to 0.001209
0 % vs 10-6 %	0,001281	2,372	P > 0.05	-0.0001177 to 0.002679

S17. Statistical analysis of eclosed flies from 80 eggs

	Paraquat concentration				
	0 mM	10^{-1} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
Number of eclosed flies	72	60	48	56	52
	68	72	40	56	58
	56	66	78	56	76
	68	62	42	42	66
	74	60	62	52	54
	46	70	70	60	40
	62	68	66	70	60
	62	64	66	64	56
	54	60	72	66	52
	54	50	64	52	42
	72	58	60	76	60
	70	44	58	68	56
	60	50	66	58	42
	68	52	56	72	42
	60	60	42	46	56
	48	54	42	56	36
	66	58	54	50	68
	54	44	48	64	48
	46	60	50	66	44
	46	48	48	68	68
	76	68	54	66	50
	60	44	44	70	48
	58	60	54	52	36
	66	42	48	50	42
	52	32	54	52	70
	68	44	60	56	60
	66	64	56	58	66
	60	48	56	58	76
	58	46	58	62	62
	60	62	48	68	66
	64	60	52	40	64
	62	52	52	48	62
	66	62	60	60	54
	62	60	70	54	62
	56	48	52	60	64
	46	64	64	60	62
	50	56	64	62	58
	66	68	58	64	54
	64	60	66	64	48
	50	56	50	52	54
	42	66	66	36	58
	52	66	74	64	66
	50	48	44	54	68
	60	46	58	42	56
	48	38	58	64	52
	46	36	66	30	46
	74	48	56	46	64
	60	48	46	42	64

One-way analysis of variance	
P value	0,3200
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	5
F	1,181
R squared	0,01970

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	0,9358
P value	0,9194
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

ANOVA Table	SS	df	MS
Treatment (between columns)	428,7	4	107,2
Residual (within columns)	21330	235	90,76
Total	21760	239	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10^{-1} mM	4,083	2,100	P > 0.05	-0.7024 to 8.869
0 mM vs 10^{-3} mM	2,667	1,371	P > 0.05	-2.119 to 7.452
0 mM vs 10^{-4} mM	2,417	1,243	P > 0.05	-2.369 to 7.202
0 mM vs 10^{-6} mM	2,917	1,500	P > 0.05	-1.869 to 7.702

S18. Statistical analysis of eclosed flies

	Paraquat concentration				
	0 mM	10^{-1} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
Number of eclosed flies	82	20	68	66	66
	70	16	58	90	48
	60	8	40	44	56
	88	24	42	88	96

One-way analysis of variance	
P value	0,0007
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	5
F	8,778
R squared	0,7007

ANOVA Table	SS	df	MS
Treatment (between columns)	9052	4	2263
Residual (within columns)	3867	15	257,8
Total	12920	19	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10^{-1} mM	58,00	5,109	P < 0.01	27.01 to 88.99
0 mM vs 10^{-3} mM	23,00	2,026	P > 0.05	-7.995 to 53.99
0 mM vs 10^{-4} mM	3,000	0,2642	P > 0.05	-27.99 to 33.99
0 mM vs 10^{-6} mM	8,500	0,7487	P > 0.05	-22.49 to 39.49

S19. Statistical analysis of AKH titre

	Paraquat concentration				
	0 mM	10^{-1} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
AKH titre	0,06598354	0,09108091	0,08496276	0,09429793	0,08408722
	0,06275306	0,04130919	0,05184843	0,06344352	0,06782658
	0,0652163	0,1008678	0,1037391	0,1214853	0,1175753
	0,06517591	0,09156338	0,1097567	0,08826458	0,08192731
	0,04765059	0,09703006	0,07917637	0,07728953	0,06241874
	0,06598354	0,1464616	0,1242787	0,1167852	0,1156324
	0,06275306	0,1661083	0,1621144	0,1436342	0,1271414
	0,0652163	0,1439384	0,1121149	0,1407367	0,1193986
	0,06517591	0,1712409	0,1436972	0,1234664	0,127077
	0,04765059	0,1468097	0,1518158	0,1122758	0,1158553
	0,06598354	0,1563299	0,1660538	0,1179286	0,06031972
	0,06275306	0,1664884	0,08601976	0,1666325	0,1160578
	0,0652163	0,03860807	0,09571436	0,08751356	0,04953915
	0,06517591	0,0618934	0,03911192	0,06301014	0,03699012
	0,04765059	0,07700641	0,07615277	0,07686413	0,08433382
	0,06598354	0,0733286	0,07677335	0,08728898	0,080000
	0,06275306	0,03602763	0,05968678	0,06261169	0,05254991
	0,0652163	0,06965933	0,05793992	0,05611691	0,05867202
	0,06517591	0,05524261	0,0530327	0,06065108	0,06438559
	0,04765059	0,07667329	0,06555033	0,08560538	0,04622844
	0,06598354		0,08142252	0,07709573	0,07987537
	0,06275306		0,06904965	0,08904667	0,081163
	0,0652163		0,05459997	0,0650514	0,09277491
	0,06517591		0,05520312	0,06927618	0,054189
	0,04765059		0,1055698	0,1006622	0,05702504
	0,06598354		0,1101007	0,07788188	0,1058502
	0,06275306		0,03132804	0,0562244	0,1390015
	0,0652163		0,05652925	0,04732782	0,04810763
	0,06517591		0,139253	0,1384621	0,1229076
	0,04765059		0,09082617	0,07307043	0,08960165
	0,06598354		0,05967856	0,05451871	0,05948979
	0,06275306		0,07849982	0,07218048	0,07370824
	0,0652163		0,05499253	0,05844815	0,05770387
	0,06517591		0,05077661	0,06623341	0,0646512
	0,04765059		0,05577917	0,06063453	0,05593584
	0,06598354		0,06141685	0,05516444	0,05385441
	0,06275306		0,06999504	0,07654861	0,07455686
			0,08444759	0,08604674	0,08049764
			0,0345323	0,0293636	0,02772787

One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	5
F	7,294
R squared	0,1331

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	94,92
P value	P<0.0001
P value summary	***
Do the variances differ signif. (P < 0.05)	Yes

ANOVA Table	SS	df	MS
Treatment (between columns)	0,03016	4	0,007541
Residual (within columns)	0,1964	190	0,001034
Total	0,2266	194	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10^{-1} mM	-0,03886	5,338	P < 0.01	-0.05681 to -0.02092
0 mM vs 10^{-3} mM	-0,02165	2,973	P < 0.05	-0.03959 to -0.003704
0 mM vs 10^{-4} mM	-0,02307	3,169	P < 0.01	-0.04102 to -0.005130
0 mM vs 10^{-6} mM	-0,01763	2,421	P > 0.05	-0.03557 to 0.0003188

S20. Statistical analysis of survival rate

	Paraquat concentration				
	0 mM	10^{-1} mM	10^{-3} mM	10^{-4} mM	10^{-6} mM
Survival (%)	42,000000	70,000000	55,000000	73,00000	75,000000
	47,000000	61,000000	72,000000	64,000000	91,000000
	32,000000	57,000000	72,000000	75,000000	68,000000
	42,000000	50,000000	60,000000	51,000000	80,000000
	50,00000	54,000000	89,000000	85,000000	65,000000

One-way analysis of variance	
P value	0,0006
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	5
F	7,835
R squared	0,6104

Bartlett's test for equal variances	
Bartlett's statistic (corrected)	2,390
P value	0,6645
P value summary	ns
Do the variances differ signif. (P < 0.05)	No

ANOVA Table	SS	df	MS
Treatment (between columns)	3440	4	860,1
Residual (within columns)	2196	20	109,8
Total	5636	24	

Dunnett's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
0 mM vs 10^{-1} mM	-15,80	2,384	P > 0.05	-33.36 to 1.761
0 mM vs 10^{-3} mM	-27,00	4,074	P < 0.01	-44.56 to -9.439
0 mM vs 10^{-4} mM	-27,00	4,074	P < 0.01	-44.56 to -9.439
0 mM vs 10^{-6} mM	-33,20	5,010	P < 0.01	-50.76 to -15.64

8. CURRICULUM VITAE

Name: Michala

Surname: Korandová

Address: Pavlovsko 74, 337 01 Rokycany

E-mail address: michalakorandova@seznam.cz

Date of birth: 12. 2. 1989

Place of birth: Rokycany

Employment:

2014 – so far

Biology centre Academy of Science Czech Republic – Institute of Entomology

Address: Branišovská 31, 370 05 České Budějovice, Czech Republic

Education:

2014 – so far

University of South Bohemia in České Budějovice – Faculty of Science

Ph.D. field: Physiology and developmental biology

Ph.D. thesis: Study of telomeric homeostasis in insects

Supervisor: RNDr. Radmila Čapková Frydrychová, Ph.D.

Consultant: prof. RNDr. Dalibor Kodrík, CSc.

2016

University of South Bohemia in České Budějovice – Faculty of Science

RNDr. field: Experimental biology

RNDr. Thesis: Effect of low doses of herbicide paraquat on antioxidant defense in *Drosophila*

Degree: RNDr.

2012 – 2014

University of South Bohemia in České Budějovice – Faculty of Science

Master field: Experimental biology – Genetics and genetic engineering

Master thesis: Transcriptional analysis of the *HeT-A* retrotransposon in *Drosophila melanogaster*

Final State master exams: Biology, Genetics, Genetics of animal – with distinction

Degree: Mgr.

2008 – 2012

University of South Bohemia in České Budějovice – Faculty of Science

Bachelor field: Biology

Bachelor thesis: Genetic variability of natural populations of the *Diuraphis noxia*

Final State bachelor exams: Genetics and Cell biology, Ecology, Zoology

Degree: Bc.

Publishing activities:

- Korandová M., Krůček T., Szakosová K., Kodrík D., Kühnlein R. P., Tomášková J., Čapková Frydrychová R. (manuscript) Chronic low-dose pro-oxidant treatment stimulates transcriptional activity of telomeric retroelements and increases telomere length in *Drosophila*

- Korandová M., Čapková Frydrychová R. (2016) Telomerase activity and telomere length in Apis mellifera. *Chromosoma* **125**: 405-411.
  DOI: 10.1007/s00412-015-0547-4

- Krucek T., Korandova M., Capkova Frydrychova R. (2015) Effect of thermal stress on telomere length in Drosophila. In: Abstracts, Twelfth International Conference on Drosophila Heterochromatin, 24-30 May 2015, Palermo, Italy (poster).

- Korandova M., Svellerova H., Krucek T., Capkova Frydrychova R. (2015) The variation in promoter activity of telomeric HeT-A element in Drosophila. In: Abstracts, Twelfth International Conference on Drosophila Heterochromatin, 24-30 May 2015, Palermo, Italy (poster).

- Krůček T., Korandová M., Szakosová K., Šerý M., Čapková Frydrychová R. (2015) Effect of low doses of herbicide paraquat on antioxidant defense in Drosophila. *Archives of Insect Biochemistry & Physiology* 88: 235–248.
  DOI: 10.1002/arch.21222

- Korandová M., Krůček T., Vrbová K., Čapková Frydrychová R. (2014) Distribution of TTAGG-specific telomerase activity in insects. *Chromosome Research* 22: 495-503.
  DOI: 10.1007/s10577-014-9436-6

Other work:

- Cooperation on the grant project Development of genetic sexing of codling moth *Cydia pomonella*, University of South Bohemia (2012 – 2014) – injication eggs of condling moth
- Supervisor: High school scientific activity – Eliška Kozáková – Vliv stresových podmínek na aktivitu telomere u *Drosophila melanogaster* (regional round of the competition)
- Teacher on week workshop: Basic methods of molecular biology
- Cooperation on the patent Protein feed containing green micro-algae for honey bee
- Cooperation on utility model Feed for honey bees

Completion of the course:

Basic methods of molecular biology (2012)

Advanced methods of molecular biology (2013)

Technology Transfer Manager - Junior (2015)

Language skills:

English – TOEFL (2013)

Doctoral State Exam from English (2016)

Germany – high school graduation (2008)

michalakorandova@seznam.cz

Telomere length compensation mechanisms as players in longevity and stress adaptation of insects.
Ph.D. Thesis, 2017

University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic
Phone: +420 387 775 054
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz