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Telomere Dynamics in Naked Mole-Rats

Master Thesis

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Submitted by

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Expression of Thanks

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Content

1. Introduction

Aging is a degenerative process that leads to a gradual deterioration of bodily functions and thereby increasing the risk of developing age-related diseases such as cancer and cardiovascular disorders as well as neurodegenerative disorders. Interestingly, some species seemingly have developed strategies during their evolution that protect them against these age-related disorders and thus, open the doors to longevity (de Medina 2019).

Whereas, humans are generally regarded as a long-lived species, there are some mammals that are even more successful in defying the aging process. Most notably, bowhead whales (*Balaena mysticetus*) – the longest living mammals on earth – which are known to have lifespans of more than 200 years (de Medina 2019). But other whale species such as blue whales, fin whales and gray whales (*Balaenoptera musculus, Balaenoptera physalus, Eschrichticus robustus*) and even some toothed whales like the narwhale (*Monodon monoceros*), too, show unusually long life-expectancies in the wild (Garde et al. 2007, Moskalev et al. 2017). Elephants (*Loxodonta africana*) are yet another example for a long-lived, but terrestrial species. Additionally, primates, whales and elephants survive well into their post-fertile stages (Lee et al. 2016).

While most long-lived mammals are of large body size, some small-sized species with exceptionally long lifespans do exist. For example, several species of microbats (e.g. *Myotis lucifugus*, *Myotis branditii*, *Eptesicus fuscus*) are known to live more than 40 years (Lambert and Portfors 2017, de Medina 2019). These microbats defy the aging process despite their high metabolic rate (Lambert and Portfors 2017). Another small-sized but long-lived exception is the naked mole-rat (*Heterocephalus glaber,* henceforward abbreviated as NMR*)* alongside other members of the Bathyergidae-family (e.g. *Fukomys anselli* and *F. mechowii*, Dammann 2017).

The NMR is a remarkable animal that puzzles and intrigues scientists all over the world since its first scientific description by Eduard Rüppell in 1842 (Hite et al. 2019, Henry et al. 2006). Endemic to arid and semiarid regions of northern Africa NMRs live in deep burrow systems below the ground. Colonies comprise up to 300 individuals that are hierarchically organized with a steep reproductive skew. Within the colony only one to two breeding females (coined queens) and up to three breeding males (pashas) exist (Henry et al. 2006). The rest of the colony members are workers and as such sexually repressed by the queen (Ruby et al. 2018). NMRs are indeed often believed to be possibly the only eusocial mammal worldwide (Ruby et

al. 2018, Bens et al. 2018). But their sociality is by far not the only astounding feature of these rodents. As subterranean dwellers they are hairless, with wrinkly skin and degenerated eyesight and are yet remarkably resistant to endogenous and exogenous threats. They are indifferent to certain types of pain, can endure hypoxic and anoxic conditions as well as high amounts of CO_2, do not fall victim to neoplastic growths and deploy an extraordinary long lifespan of more than 30 years (Lewis and Buffenstein 2016).

This depiction of longevity is especially astounding given the fact that NMRs are mouse-sized rodents that weigh on average only around 40 g per animal (Blagosklonny 2013). In fact, based on their body size the estimated lifespan of an NMR should not exceed six years (Lewis et al. 2018). But not only do they contradict the correlation between body size and longevity (Blagosklonny 2013). They also defy the disposable soma theory of aging which would allow an organism either to use its energetic reserves to maintain its cellular functions resulting in a longer life expectancy or deploy its resources to gain high reproductive rates. However, NMR queens pose a contradiction to this theory since they are the only females that reproduce within their colony and at the same time are often believed to be the oldest individuals therein (Bens et al. 2018). Indeed, breeders of closely related *Fukomys sp.* have been found to live longer than non-breeding colony members (Dammann 2017). This lifespan difference between workers and queens is a rare feature in mammals, but otherwise often seen in eusocial insects. It is believed, that sociality there modifies the aging process and thereby enabling distinctive aging patterns between workers and queens. Queens of eusocial insects often display a life expectancy that is 60 times higher compared to the life expectancy of workers. Furthermore, these distinctive aging patterns might not solely be a result of different lifestyles with workers engaging in more risk-associated tasks (e.g. defending the nest, foraging for food) and queens remaining inside the nest and focusing on reproduction whilst relying on the colony to safeguard them, but also of the inherent quality of the individual to defy the energetic demands of reproduction and at the same time realizing its potential of longevity (Blacher et al. 2017).

Moreover, NMRs not only exhibit exceptional longevity they also display no signs of increased age-dependent mortality until well into their twenties. Nor do they enter cellular senescence until late in life and are thereby maintaining a non-aging phenotype with queens being able to reproduce until their end of life (Lewis et al. 2018).

Cells that enter senescence are locked in a state of cell cycle arrest and permanently lose their ability to proliferate accompanied by a variety of changes that affect their morphology as well

as their biochemical functions (Bernadotte et al. 2016). Since long telomeres are often believed to shield an individual from the onset of cellular senescence (Haussmann and Mauck 2008), telomeres are proposed markers of aging (Bernadotte et al. 2016).

Telomeres are a common feature found in all eukaryotic organisms. They are complexes of proteins and short nucleotide sequences composed of TTAGGG repeats, that encapsulate the ends of chromosomes (Bernadotte et al. 2016). Since the DNA polymerase cannot replicate DNA in a 3'-direction, the end-sequences get trimmed with every mitotic cycle (Haussmann and Mauck, 2008, Bernadotte et al. 2016) - a phenomenon also known as the end replication problem (Haussmann and Mauck, 2008). Thus, telomeres gradually shorten with time until reaching a critical state upon which cells are triggered into replicative senescence and cease to divide. Furthermore, short telomeres are associated with higher mortality and decreased longevity – when comparing similarly aged individuals of the same species (Haussmann and Mauck, 2008). Nonetheless, telomeres do not only shorten, but can also elongate with time – as has been found in other mammalian species (Hoelzl et al. 2016, van Lieshout et al. 2019). This process of telomere maintenance is facilitated by the enzyme telomerase. It is plausible that telomerase activity and thereby telomere maintenance and lengthening with age could be determined by individual inherent characteristics and differ between members of the same species (Haussmann and Mauck, 2008). Thus, it is conceivable that NMR queens show a different pattern of telomere dynamics than NMR workers.

When it comes to testing telomere dynamics and telomere length, there are three main approaches that are used – Southern blots, flow fluorescence in situ hybridization (flow FISH), and quantitative polymerase chain reaction (qPCR). With Southern blots, the length of terminal restriction fragments is measured and compared. This comprises two disadvantages: the analysis relies on samples with a high DNA-yield and often results in an overestimation of telomere length, since it does not omit the subtelomeric region (Wang et al. 2018). Flow FISH on the other hand, uses fluorescent-labeled peptide nucleic acid probes that specifically bind to telomere repeats combined with flow cytometry. As such, the measurement is conducted on the single cell level and has the potential for highly accurate results (Wand et al. 2016). Yet, on the downside this method is highly technical and therefore cost-intensive. Therefore, the qPCR method, established by Cawthon (2002), has become the most commonly used technique to measure telomere length – with it, the relative ratio of the amplified telomeric sequence to the amplified sequence of a non-variable copy-number gene is calculated (Vasilishina et al. 2019; Wang et al. 2018, Cawthon 2002). The resulting ratio enables

conclusions to be drawn concerning the average telomere length within a sample (Vasilishina et al. 2019). The qPCR method allows for high throughput as well as low DNA-content in the original sample (Wang et al. 2018). Furthermore, it has been successfully used for a vast variety of different species and existing protocols can be easily adapted to measure relative telomere length in most mammalian species (Morinha et al. 2020) – due to the homologous nature of telomere sequences (Bernadotte et al. 2016).

However, if one was to set out to study and compare the biology of NMRs, there is yet another obstacle to overcome. Namely, that it is hard to tell the sexes apart. NMRs only enter puberty when becoming reproducing members of the colony (Bens et al. 2018). Therefore, only pashas and queens show easily distinguishable sexual characteristics (Jarvis 1991). Thus, a molecular approach constitutes an elegant tool to accurately differentiate between male and female animals. Molecular sexing approaches are DNA-based and rely on genetic sequences that are specific for X or Y chromosomes. These can easily be amplified using polymerase chain reaction (PCR) methods and made visible with agarose gel electrophoresis.

1.1 Aims of This Thesis

I. Establishing a PCR-based molecular sexing protocol for NMRs.
II. Establishing a protocol for relative telomere length measurement in NMRs using a qPCR-approach.
III. Elucidating whether there is a difference in telomere dynamics over a certain time period (ranging from 6 to 12 month) in NMRs of different social status and sex and in dependence of their monthly weight gain.

2. Materials & Methods

2.1 Study Population

In total 89 NMRs participated in this study. These animals were housed in five different colonies (A, B, C, D, H) at Zoo Schönbrunn in Vienna. The colonies comprised of twelve to 21 animals each. Colony A and B were located in the Wüstenhaus and part of the exhibition there. Colony C, D and H were not exhibited and located in another building without public access. The animals were kept in tube-systems (3.2-6.9 m) made from glass or plexiglass-tubes (~ 7 cm in diameter) and cubic glass-chambers (~ 25 x 25 cm) that served as nesting-, feeding- or defecating-chambers. The rooms in which the animals were kept were constantly heated and maintained at a temperature of 30 °C. The animals were fed *ad libitum* once a day with a variety of fresh vegetables. In addition, gnawing materials (twigs) were offered. Wood chips and straw were provided as litter and nesting material. The tube-systems were cleaned once a day – the used litter and nesting material together with uneaten fruits and vegetables was replaced with new items.

2.2 Transponder Fitting

All animals that participated in the study were chipped with Radio-frequency identification-tags (RFID-tags) for individual identification by the zoo's veterinarians. Animals that lost their RFID-tags during the course of the study were refitted with tags.

2.3 Sampling

DNA used for molecular sexing as well as relative telomere length measurements was extracted from buccal mucosa cells. To this end, cell samples were taken twice during the study and sampling intervals were kept between six and twelve months. Before sampling, each animal was identified by its tag-number and its weight was taken, then a brush (Gynobrush®, Heinz Herenz Medizinalbedarf, Hamburg, Germany) was inserted into its mouth and twirled for five to ten seconds at the inside of its cheek to harvest buccal mucosa cells. The brush was then placed in an empty 1.5 mL Eppendorf tube, the extending brush stick was cut to fit the tube and the tube was sealed. Samples were kept on ice (at 4 °C) during transport and stored at -20 °C until processing.

2.4 DNA-Extraction

Genomic DNA was extracted from the buccal mucosa samples using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany, Cat No./ID: 69504) for cells and tissue following the manufacturer's protocol – with the following exceptions: After adding the second wash buffer (AW2), samples were centrifuged at 13200 rpm. The flow through was discarded, the collection columns were placed in 1.5 mL Eppendorf tubes and 100 µL of elution buffer (AE) were added. Samples were then incubated for 5 to 10 min at room temperature, afterwards centrifuged at 8000 rpm for 1 min and the eluate was collected and stored at -20 °C until further use.

The resulting DNA-concentration of each sample was measured using a spectrophorometer (NanoDrop™ 2000, Thermo Scientific™, Waltham, Massachusetts, USA) to guaranty a sufficient DNA-yield of ≥ 5 ng/µL. To this end, 1 µL of DNA-solution was applied on the machine's pedestal and measured according to the manufacturer's protocol. DNA-extraction buffer was used as a blank.

2.5 Molecular Sexing

2.5.1 Protocol Development

A molecular sexing protocol was developed to identify the sex of each animal. To this end, a multiplex PCR approach, comprising two sets of forward and reverse primers, had to be established. These primer sets consisted of sexing primers, that only and specifically amplify a gene sequence located on the male Y chromosome and a control set, that equally works on somatic DNA of both males and females to ensure that the PCR process is working correctly, and amplification takes place (internal positive control). In our case, forward and reverse primers that amplify the *Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* gene were used as internal positive control (5'-CAG CCT TGG GCA GGT TTG TG-3' and 5'-CAC AAA CAT GGG GGC ATC CG-3', respectively). The sexing primers were designed to specifically amplify a zinc finger-motif located on the NMR's Y chromosome and comprised the following forward primer (5′-TTT TCC GTG TAG AAA GGG TTT-3′) and reverse primer (5′-TTG TGA CTC GCC ACC CTT TC-3′).

The first step in developing a molecular sexing protocol consisted of a gradient PCR including seven different annealing temperatures to find the temperature were both primer sets can be used simultaneously within the same reaction mixture, whilst resulting in the highest possible

number of amplificants for both primer sets. This temperature would later be used for the subsequent multiplex PCR measurements. Concurrently, these tests should ensure that the used sexing primers indeed act only on the gene located on the Y chromosome.

To this end, 4 reaction mixtures containing FIREPol DNA polymerase (Solis BioDyne, Tartu, Estonia) were prepared with DNA obtained from 2 NMRs of known sex (one male, one female) as depicted in tab.1. A negative control was prepared in addition. Each reaction mixture was then vortexed for 5 s and split into seven 200 µL PCR tubes – one for each tested temperature.

Tab. 1: Reaction mixtures used for molecular sexing within the protocol development

	Reaction mixture 1 (per 200 µL PCR tube)	Reaction mixture 2 (per 200 µL PCR tube)	Reaction mixture 3 (per 200 µL PCR tube)	Reaction mixture 4 (per 200 µL PCR tube)	Negative control (per 200 µL PCR tube)
FIREPol DNA polymerase (5 U/µL)	0.2 µL	0.2 µL	0.2 µL	0.2 µL	0.2 µL
10x Reaction buffer (0.8M Tris-HCl, 0.2M $(NH_4)_2SO_4$, 0.2% w/v Tween-20)	1 µL	1 µL	1 µL	1 µL	1 µL
$MgCl_2$ (25 mM)	1 µL	1 µL	1 µL	1 µL	1 µL
dNTPs (10 mM)	0.5 µL	0.5 µL	0.5 µL	0.5 µL	0.5 µL
Aqua destillata	5.7 µL	5.7 µL	5.7 µL	5.7 µL	6 µL
Sexing primer forward	0.3 µL	0.3 µL	-	-	-
Sexing primer reverse	0.3 µL	0.3 µL	-	-	-
GAPDH primer forward	-	-	0.3 µL	0.3 µL	-
GAPDH primer reverse	-	-	0.3 µL	0.3 µL	-
Sample DNA (♀)	1 µL	-	1 µL	-	-
Sample DNA (♂)	-	1 µL	-	1 µL	-

Four different reaction mixtures were prepared. Two with male DNA containing either GAPDH-primers or sexing primers and analogously two with female DNA. The negative control contained neither primers nor DNA.

The PCR tubes were then spun down and a gradient PCR was performed – a detailed cycle description is given in tab. 2. Annealing temperatures ranged from 52 to 62 °C (52, 52.9, 54.3, 56, 58.3, 60.1 and 62 °C, respectively).

Tab. 2: Gradient PCR cycle description in molecular sexing protocol-development

	Temperature	Duration	
Enzyme activation (FIREPol DNA polymerase)	95 °C	5 min	
Denaturation	95 °C	40 s	Cycling = 30x
Primer annealing	52 °C to 62 °C	35 s	
Elongation	72 °C	35 s	
Final elongation	72 °C	7 min	
Rest	12 °C	∞	

Cycling consisted of 5 min at 95 °C, followed by 30 cycles of 40 s at 95 °C, 35 s at 52 to 62 °C, 35 s at 72 °C, followed by a final elongation step at 72 °C for 7min and an infinite hold at 12 °C.

After completion 8 µL of the resulting PCR products from each sample were mixed with 2 µL DNA gel loading dye (6x, Thermo Scientific™, Waltham, Massachusetts, USA) and loaded onto a 2% agarose gel stained with 4 µL GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California, USA) and gel electrophoresis was performed at a current of 120 V. DNA-bands were subsequently detected under UV-light.

2.5.2 Molecular Sexing with Multiplex PCR

After successfully establishing a molecular sexing protocol as described above, all 85 NMRs that participated in the study were sexed using the multiplex PCR approach. To this end, a premixed PCR master mix containing FIREPol DNA polymerase (Solis BioDyne, Tartu, Estonia) was used to which primers (forward and reverse sexing primers plus forward and reverse GAPDH-primers) and distilled water were added. GAPDH-primers served as an internal positive control. This mixture was vortexed for 5 s and split into 200 µL PCR tubes to which 1 µL of sample DNA was then added. All samples were prepared in duplicates. A reaction mixture in which distilled water was used instead of DNA served as a negative control. See tab. 3 for the exact sample preparation. Before starting the PCR process, all samples were spun down. A detailed PCR cycle description is given tab. 4.

Tab. 3: Sample preparation scheme for molecular sexing by multiplex PCR

	Reaction mixture (per 200 µL PCR tube)	Negative control (per 200 µL PCR tube)
Master mix containing FIREPol DNA polymerase (5 U/µL)	5 µL	5 µL
Aqua destillata	2.8 µL	3.8 µL
Sexing primer forward (10 µM)	0.3 µL	0.3 µL
Sexing primer reverse (10 µM)	0.3 µL	0.3 µL
GAPDH primer forward (10 µM)	0.3 µL	0.3 µL
GAPDH primer reverse (10 µM)	0.3 µL	0.3 µL
Sample DNA	1 µL	-

To the FIREPol DNA polymerase master mix primers (forward and reverse sexing primers plus forward and reverse GAPDH-primers) and distilled water were added. This mixture was vortexed for 5 s and split into 20 µL PCR tubes to which 1 µL of sample DNA was added. The resulting reaction mixture per tube is shown in this table. The negative control contained distilled water instead of DNA.

Tab. 4: Multiplex PCR cycle description for molecular sexing

	Temperature	Duration	
Enzyme activation (FIREPol DNA polymerase)	95 °C	15 min	
Denaturation	95 °C	40 s	⎫
Primer annealing	54.3 °C	35 s	⎬ Cycling = 30x
Elongation	72 °C	35 s	⎭
Final elongation	72 °C	7 min	
Rest	12 °C	∞	

After 15 min at 95 °C to activate the FIREPol DNA polymerase, 30 cycles were executed consisting of 40 s at 95 °C, 35 s at 54.3 °C, 35 s at 72 °C, followed by a final elongation step at 72 °C for 7min and an infinite hold at 12 °C.

After completion, 8 µL of the resulting PCR products from each sample were mixed with 2 µL DNA gel loading dye (6x, Thermo Scientific™, Waltham, Massachusetts, USA) and loaded onto a 2% agarose gel stained with 4 µL GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California, USA) and gel electrophoresis was performed at a current of 120 V. DNA-bands were subsequently detected under UV-light.

2.6 Measuring Telomere Dynamics with qPCR

A qPCR protocol was developed for NMRs based on the Cawthon method. There the T/S ratio of a reference sample is compared to the T/S ratio in the experimental sample while the number of cycles is kept constant. Thus, T/S=1 is met, when the experimental DNA is equivalent to the reference sample in regard to the amplified telomere repeats as well as the amplified non-variable copy-number gene (also referred to as single copy-number gene). Hence, the relative telomere length of one individual should resemble the difference between the T/S ratio found in the reference sample compared to the T/S ratio of the sample DNA (Cawthon, 2002).

Preparation of reference samples: Genomic DNA samples of 4 NMRs – including one queen - were pooled. The pooled reference sample was then diluted to a DNA concentration of 5 ng/µL with distilled H_2O. A second – tissue derived – reference sample was analogously prepared and used as an internal standard. Both reference sample and internal standard were stored at -20 °C.

Experimental sample preparation: Two genomic DNA samples derived from 50 animals (10 randomly chosen animals from each colony, including one queen from each colony) obtained at two distinct time points were diluted with distilled H_2O to a DNA concentration of 5 ng/100 µL. The diluted samples were then stored at -20 °C.

Tel1b (5´-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3´) and Tel2b (5´-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3´) primers (O'Callaghan et al. 2018) were used to amplify the telomeric repeats. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was selected as the non-variable copy-number gene, thus GAPDH forward (5´-CAG CCT TGG GCA GGT TTG TG-3´) and reverse primers (5´-CAC AAA CAT GGG GGC ATC CG-3´) were used for the amplification process.

The measurements were conducted with a Rotor-Gene Q (Qiagen, Hilden, Germany) PCR cycler and non-baseline corrected raw data was analyzed with the LinRegPCR (2012.0) software package. To obtain the relative telomere length for each sample the formula adapted by Hölzl et al. (2016) was used, which is shown below.

$$RTL = \frac{E_T^{C_{tT}} / E_{ST}^{C_{tST}}}{E_C^{C_{tC}} / E_{SC}^{C_{tSC}}}$$

2.6.1 Establishing a qPCR Protocol for Relative Telomere Length

2.6.1.1 Gradient PCR

A gradient PCR with telomere primers was conducted to find the annealing temperature resulting in the highest amount of PCR products. To this end, five different annealing temperatures were tested ranging from 56 °C, 58.3 °C, 60 °C, 62.3 °C, to 64.1 °C, respectively.

The experimental sample DNA was extracted from buccal mucosa samples as described before. Three samples from colony A were randomly chosen and diluted with distilled H_2O to a DNA concentration of 5 ng/100 µL. In each run a negative control was also carried along, that did not contain any DNA.

Tel1b (5´-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3´) and Tel2b (5´-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3´) primers (O'Callaghan et al. 2018) were used as forward and reverse primer, respectively to bind to the telomeric repeats within the sample DNA. GoTaq® qPCR Master Mix (Promega, Madison, Wisconsin, USA) containing GoTaq® Hot Start DNA polymerase was used. A depiction of the sample preparation is shown in tab. 5.

Tab. 5: Sample preparation for gradient PCR with Tel1b and Tel2b primers

	Reaction mixture (per 200 µL PCR tube)	Negative control (per 200 µL PCR tube)
GoTaq® qPCR Master Mix (Promega)	5 µL	5 µL
Aqua destillata	2.4 µL	4.4 µL
Tel1b (10 µM)	0.3 µL	0.3 µL
Tel2b (10 µM)	0.3 µL	0.3 µL
Sample DNA (5 ng/100 µL)	2 µL	-

Sample DNA consisted of three samples from colony A, that were randomly chosen and diluted with distilled H_2O to a final DNA concentration of 5 ng/100µL. A premix with GoTaq® qPCR Master Mix (Promega) containing GoTaq® Hot Start DNA polymerase was prepared to which Tel1b and Tel2b telomere primers and distilled water were added. This mixture was vortexed for 5 s and split into 20 µL PCR tubes to which 1 µL of sample DNA was added. The resulting reaction mixture per tube is shown in this table. The negative control contained distilled water instead of DNA.

Before transferring the samples into the PCR machine, all samples were vortexed for 5 s and subsequently spun down. A detailed cycle description is shown in tab. 6.

Tab.6: Gradient PCR cycle description for Tel1b and Tel2b primers

	Temperature	Duration	
Enzyme activation	95 °C	2 min	
Denaturation	95 °C	20 s	Cycling = 40x
Primer annealing	56 °C to 64.1 °C	20 s	
Elongation	72 °C	20 s	
Final elongation	72 °C	1 min	
Rest	12 °C	∞	

After 2 min at 95 °C of enzyme activation, 40 cycles were executed consisting of 20 s at 95 °C, 20 s at 56 to 64.1 °C, 20 s at 72 °C, followed by a final elongation step at 72 °C for 1 min and an infinite hold at 12 °C.

After completion 8 µL of the resulting PCR products from each sample were mixed with 2 µL DNA gel loading dye (6x, Thermo Scientific™, Waltham, Massachusetts, USA), loaded onto a 2% agarose gel stained with 4 µL GelRed Nucleic Acid Gel Stain (Biotium, Hayward, California, USA) and gel electrophoresis was performed at a current of 120 V. DNA-bands were subsequently detected under UV-light.

2.6.1.2 qPCR Test Runs

Test runs were conducted with GAPDH primers at annealing temperatures of 62 and 64 °C, respectively and with Tel1b and Tel2b primers at 58 and 60 °C, respectively to optimize the PCR efficiency (≥ 0.8). Additionally, with the Tel1b and Tel2b primers the pipetting scheme was altered using 1.2 µL instead of 0.6 µL of primers and an additional 2 µL of dNTPs (10 mM).

2.6.2 Measuring Relative Telomere Length in NMRs with qPCR

Following the protocol development described in 3.6.1, the diluted genomic DNA samples (5 ng/100 µL) from all 50 animals were subsequently measured. Telomere PCR runs were always

followed by non-variable copy-number gene PCR runs of the same samples. Sample position in the rotor between runs was kept constant. All samples were measured in triplicates.

GAPDH was used as a non-variable copy-number gene. The pipetting scheme for GAPDH runs and telomere runs, respectively are shown in tab. 7 and 8.. Cycling conditions of the qPCR runs correspond to the description shown in tab. 7., except for the primer annealing temperatures. Annealing temperature for GAPDH primers was 64 °C and 60 °C for telomere primers.

Tab. 7: Reaction mixtures for qPCR runs with GAPDH-primers

	Reaction mixture (per 200 µL PCR tube)	Negative control (per 200 µL PCR tube)
GoTaq® qPCR Master Mix (Promega)	5 µL	5 µL
Aqua destillata	1.8 µL	3.8 µL
GAPDH forward primer (10 µM)	0.6 µL	0.6 µL
GAPDH reverse primer (10 µM)	0.6 µL	0.6 µL
Sample DNA (5 ng/100 µL)	2 µL	-

Sample DNA was diluted with distilled H_2O to a final DNA concentration of 5 ng/100 µL. A premix with GoTaq® qPCR Master Mix (Promega) containing GoTaq® Hot Start DNA polymerase was prepared to which GAPDH primers and distilled water were added. This mixture was vortexed for 5 s and split into 20 µL PCR tubes to which 2 µL of sample DNA were added. The resulting reaction mixture per tube is shown in this table. The negative control contained distilled water instead of DNA.

Tab. 8: Reaction mixture for qPCR runs with Tel1b and Tel2b primers

	Reaction mixture (per 200 µL PCR tube)	Negative control (per 200 µL PCR tube)
GoTaq® qPCR Master Mix (Promega)	5 µL	5 µL
Aqua destillata	-	2 µL
Tel1b primer (10µM)	1.2 µL	1.2 µL
Tel2b primer (10µM)	1.2 µL	1.2 µL
Sample DNA (5ng/100 µL)	2 µL	-
dNTPs (10mM)	0.6 µL	0.6 µL

Sample DNA was diluted with distilled H_2O to a final DNA concentration of 5 ng/100 µL. A premix with GoTaq® qPCR Master Mix (Promega) containing GoTaq® Hot Start DNA polymerase was prepared to which Tel1b and Tel2b telomere primers, distilled water and additionally, dNTPs (10 mM) were added. This mixture was vortexed for 5s and split into 20 µL PCR tubes to which 2 µL of sample DNA were added. The resulting reaction mixture per tube is shown in this table. The negative control contained distilled water instead of DNA.

2.6.3 Statistics

Relative telomere length at two time points was measured and compared for each animal to elucidate changes in individuals over a certain time period. All statistical analyzes were conducted in R 3.5.1 using the package lme4 to calculate a mixed linear model. Predictor variables were sex, social status (queen or worker) and bodyweight. The sample size was limited to 49 animals (n=49) – the data of one animal had to be excluded from the calculation due to irregularities in its qPCR run.

3. Results

3.1 Molecular Sexing

3.1.1 Protocol Development

A gradient PCR with two sets of primers at seven different annealing temperatures (ranging from 52 to 62 °C) followed by gel electrophoresis of the resulting PCR products was conducted to find out, which annealing temperature could be used successfully for both primer sets. Furthermore, the results should ensure that the sexing primers were specific for the Y chromosome. The primer sets consisted of forward and reverse primers for the GAPDH gene and sexing primers that were designed to specifically amplify a zinc finger-motif on the male Y chromosome. GAPDH-primers were intended as an internal positive control and should give equal results for both female and male NMR DNA.

As would be expected GAPDH-primers equally amplified the corresponding gene sequence on male and female DNA – resulting in slightly brighter bands on the agarose gels at lower annealing temperatures. However, amplification took place at all tested temperatures. These results are shown in fig. 1 and fig. 2.

The sexing primers were shown to specifically amplify the corresponding genetic sequence on the male Y-chromosome. Still, amplification took only place at the lower annealing temperatures (52 °C, 52.9 °C and 54.3 °C, respectively). These results are depicted in fig. 3 and fig. 4. Therefore, it was decided to subsequently use an annealing temperature of 54.3 °C for the future multiplex PCR set-up, where both primers were used simultaneously in one reaction mixture.

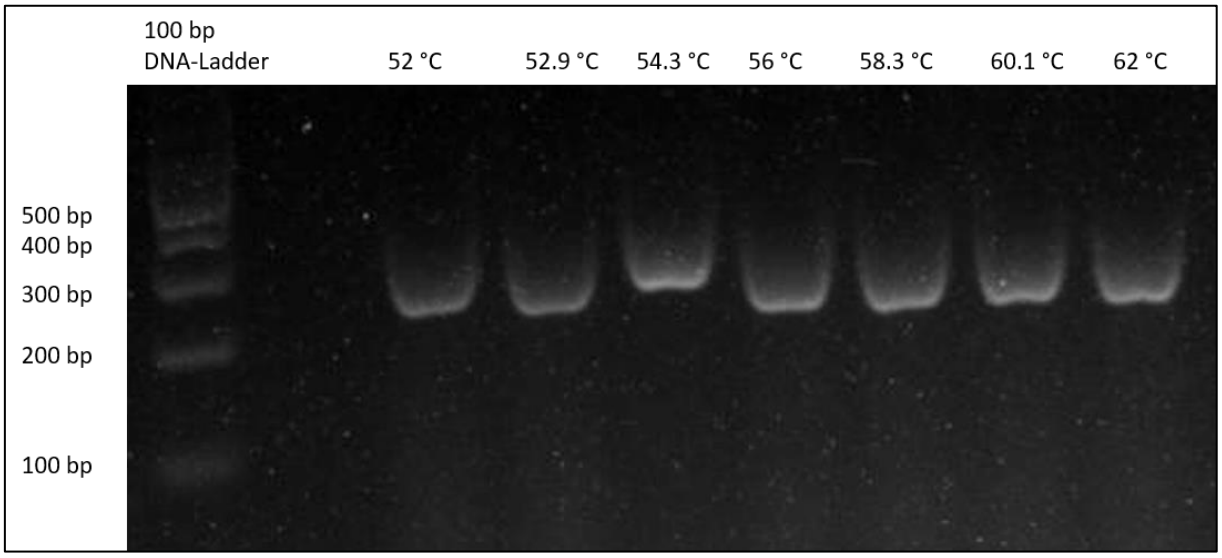

Fig. 1: Gradient PCR with GAPDH-primers using male NMR DNA

Annealing and DNA amplification took place at all seven annealing temperatures. However, the brightest band was obtained at 54.3 °C.

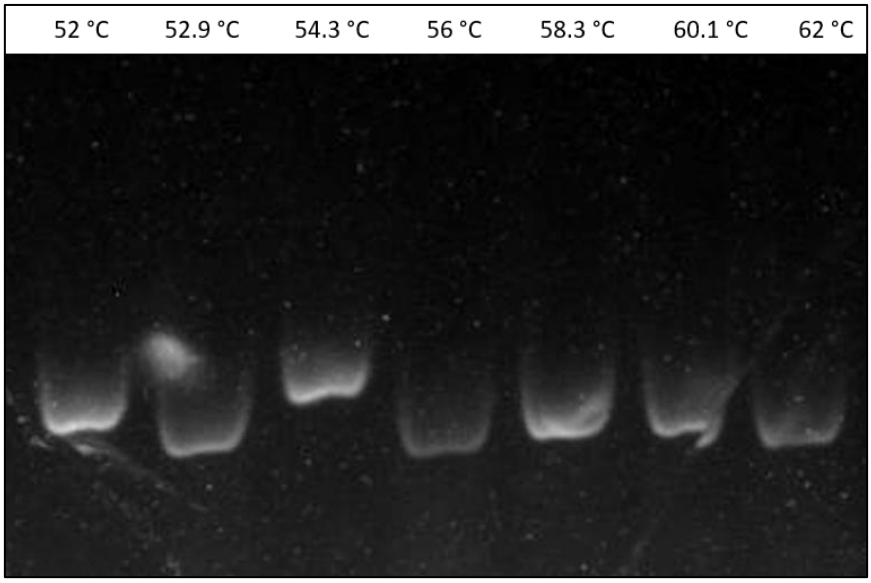

Fig. 2: Gradient PCR with GAPDH-primers using female NMR DNA

As with the male DNA annealing and amplification took place at all seven annealing temperatures. However, the brightest band was obtained at 54.3 °C.

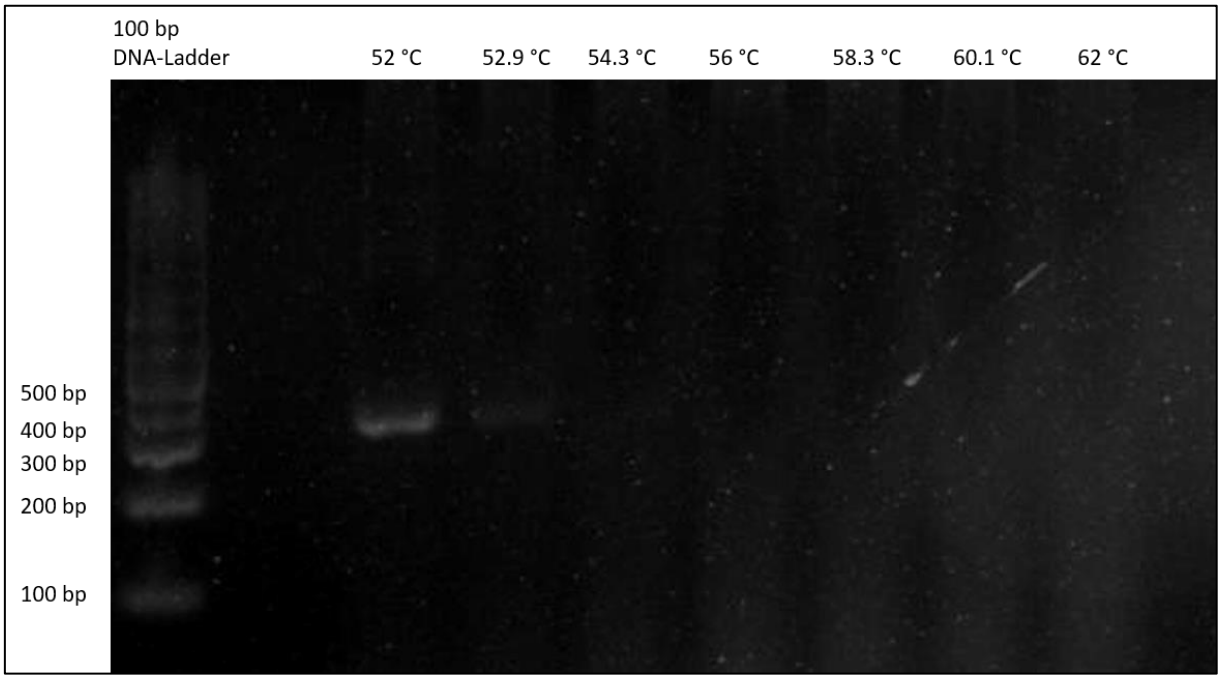

Fig. 3: Gradient PCR with sexing-primers using female NMR DNA

No annealing or amplification can be observed for any of the tested temperatures on female DNA. (The band appearing at 52 °C between 300 and 400 bp is most likely a primer dimer or some other contamination.)

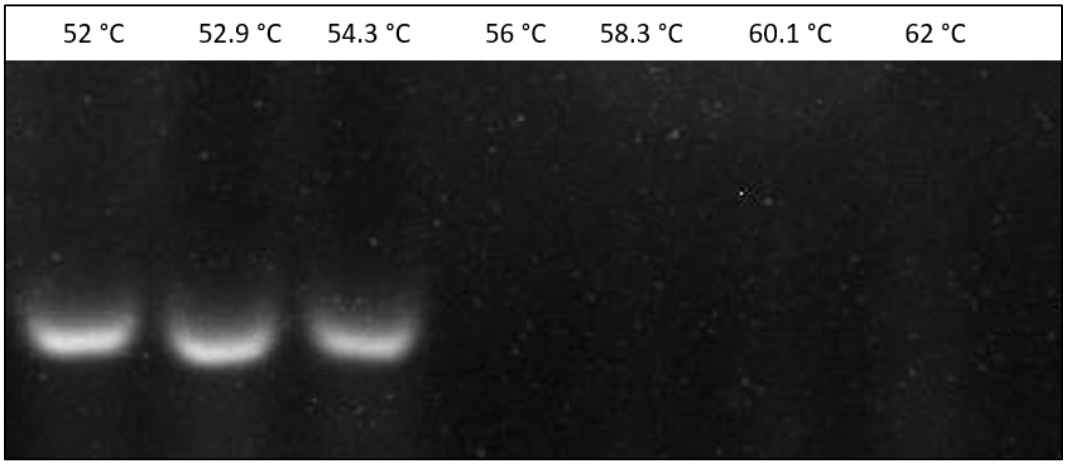

Fig. 4: Gradient PCR with sexing-primers using male NMR DNA

As expected, annealing and amplification can be observed, although only at the lower temperatures (52, 52.9 and 54.3 °C, respectively).

3.1.2 Molecular Sexing with Multiplex PCR

85 animals that participated in the study were successfully sexed using the multiplex PCR approach previously described. An excerpt of the results obtained is shown in fig. 5. In total, 27 (or ~32%) of the animals were females and 58 (or ~68%) were males – resulting in a female to male sex ratio of 1:2.15 as depicted in fig. 6.

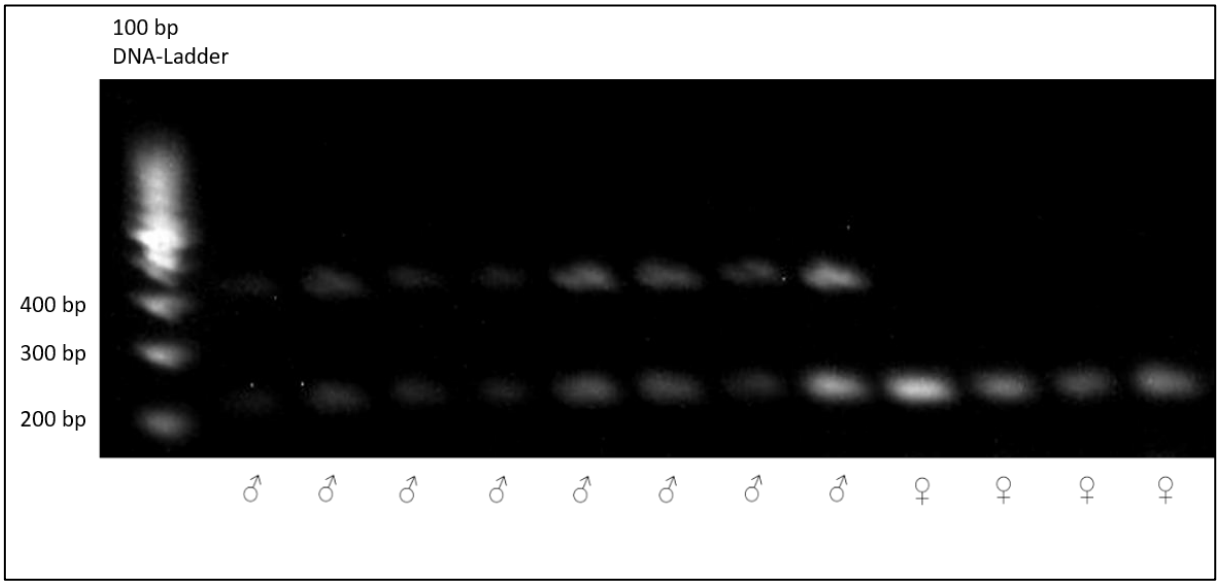

Fig. 5.: Example of a Sexing PCR result

The amplification of male DNA results in two bands when PCR products are loaded onto an agarose gel and separated via gel electrophoresis. The upper band here corresponds to a DNA-sequence located on the Y chromosome. While the bottom band links to the PCR product corresponding to the *GAPDH*-gene that is found both in males and females. It serves as a positive control ensuring that the PCR-process is working correctly. Thus, male sex will result in two bands on the gel, while female sex will result in only one.

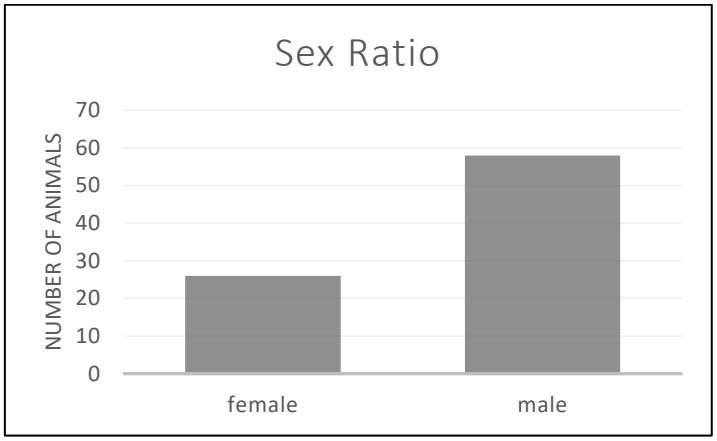

Fig. 6.: Male to Female Sex Ratio

Molecular sexing of 85 animals revealed a female to male sex ratio of 1:2.15.

3.2 Measuring Telomere Dynamics with qPCR

3.2.1 Establishing a qPCR Protocol to Measure Relative Telomere Length

A gradient PCR with telomere primers (Tel1b and Tel2b) at five different annealing temperatures – ranging from 56 to 64 °C - followed by gel electrophoresis of the resulting PCR products was conducted to find out which annealing temperature could be used successfully for the following qPCR measurements. With annealing temperatures of 64 °C and 62 °C no PCR products were detectable on the gel for one of three samples. The brightest signals were detected with annealing temperatures of 58 °C and 60 °C, respectively for all three tested samples - as can be observed in fig. 7. Hence, it was decided to use both annealing temperatures (58 °C and 60 °C) for Tel1b and Tel2b primers in the subsequent qPCR test runs.

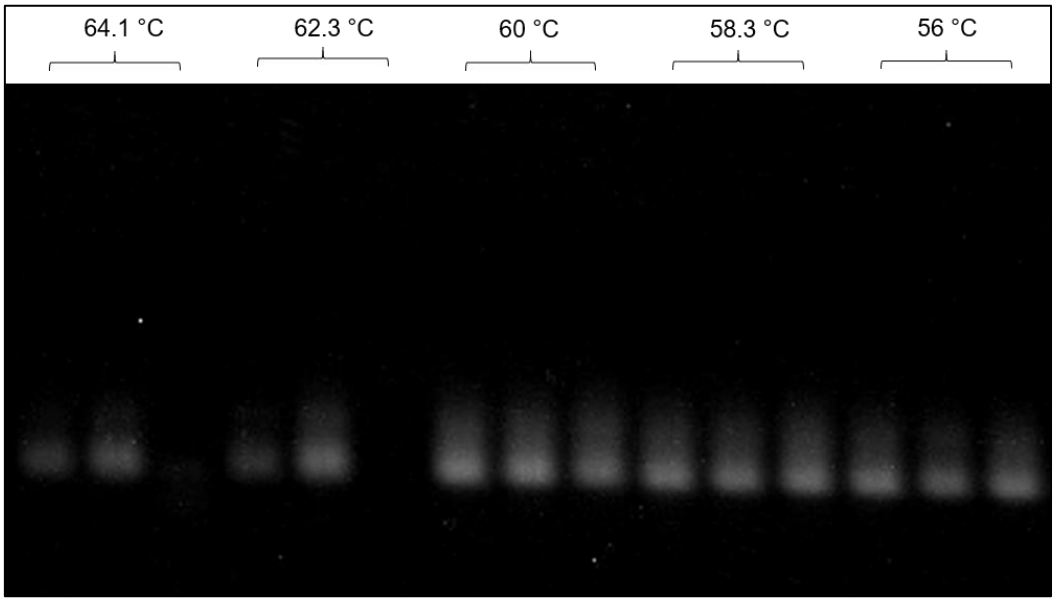

Fig. 7.: Gradient PCR with Tel1b and Tel2b primers

A gradient PCR was performed with Tel1b and Tel2b primers to find the optimal annealing temperatures for the subsequent qPCR to measure relative telomere length.

In the next step, qPCR efficiency was adjusted. To this end, qPCR test runs using Tel1b and Tel2b primers to amplify the telomere sequence and GAPDH primers to amplify the GAPDH gene that was used as the non-variable copy-number gene were conducted for two different annealing temperatures each. Telomere primers were tested at 58 °C and 60 °C, respectively. GAPDH-primers were tested at 62 °C and 64 °C, respectively. Furthermore, a test run with a doubled amount of primers and additionally added dNTPs was performed for telomere primers. The highest qPCR efficiency (Ct ≥ 1.8) was achieved with an annealing temperature of 60 °C, a doubled amount of primers and additional dNTPs (2 µL of a 10 mM dNTP solution) added to the qPCR master mix for telomere primers. On the other hand, qPCR test runs for GAPDH primers showed the highest efficiency (Ct ≥ 1.8) at an annealing temperature of 64 °C. Hence, it was decided to go forth with the following cycling conditions for GAPDH and telomere runs, respectively: GAPDH run: 2 min at 95 °C, followed by 40 cycles of 20 s at 95 °C, 20 s at 64 °C, and 20 s at 72 °C. Telomere run: 2 min at 95 °C, followed by 40 cycles of 20 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C. At the end of each run a final melting step was performed where the temperature subsequently increased by 1 °C starting with 65 °C and ending with 95 °C.

3.2.2 Relative Telomere Length in NMRs

After the establishment of a qPCR protocol to measure relative telomere length in NMRs, 100 genomic DNA samples from 50 different animals taken at two distinct timepoints were measured with qPCR to obtain the relative telomere length.

Mean qPCR efficiencies (Ct) for telomere runs reached 90% and 89% for non-variable copy number gene runs, respectively. The inter-assay variation (mean coefficient of variation) for Ct values was 0.5% for telomere runs and 0.9% for non-variable copy number gene runs.

3.2.3 Statistics

Changes in relative telomere length per month were unrelated to the animals' sex, social status or weight change per month as shown in tab. 9. Social status had no significant influence on telomere dynamics (p=0.61) and both queens and workers showed a minimal decline in relative telomere length over time. Furthermore, telomere dynamics did not alter significantly between male and female NMRs (p=0.86). These results are depicted in fig. 8 and 9, respectively. Neither did growth (expressed as weight change) influence telomere dynamics significantly over time (p=0.2) – although a trend might be deduced here. The change in relative telomere length and weight over time is depicted in fig. 10. Within the whole sample population (n=49) the mean T/S-ratio was 0.82 ± 0.30 at the first point of sampling and 0.69 ± 0.45 at the second point of sampling, respectively. Resulting in a mean monthly change in T/S-ratio of -0.01 ± 0.04, which is statistically significant (p=0.02).

Tab. 9: Estimates and Standard Errors for Changes in Relative Telomere Length

Changes in Relative Telomere Length per Month		
Predictors	**Estimate**	**Standard Error**
Intercept	0.001443	0.020300
Sex	-0.002351	0.012878
Social Status	-0.009704	0.021812
Weight Change	-0.008012	0.006451

Coefficients (estimate) and standard errors obtained from the mixed linear model are shown. Sample size of all calculations is 49 animals of five different colonies. P-values are not displayed.

Fig. 8.: Telomere Dynamics in Queens and Workers

Telomere dynamics expressed in Change in T/S-Ratio per Month did not differ significantly between queens and workers (p=0.61). Both showed a decline in relative telomere length over time.

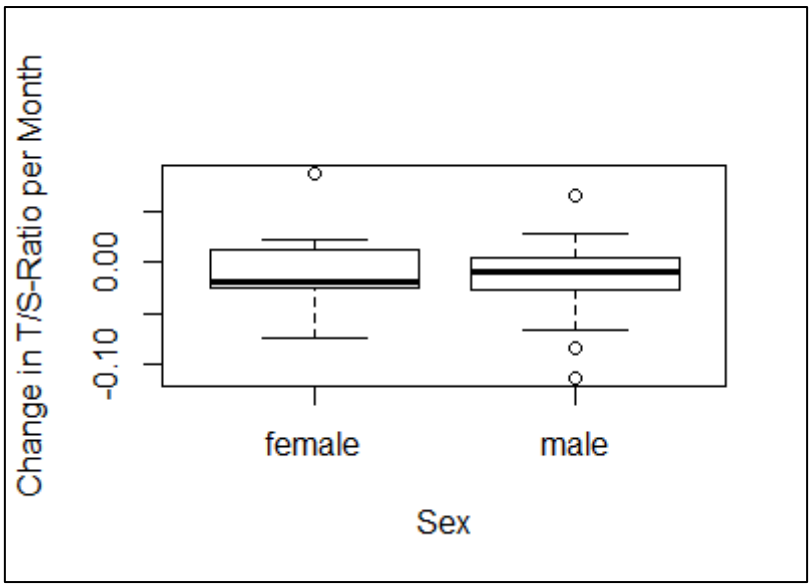

Fig. 9.: Telomere Dynamics in Male and Female NMRs

Telomere dynamics expressed in Change in T/S-Ratio per Month did not differ significantly between the sexes (p=0.86). Both showed a decline in relative telomere length over time.

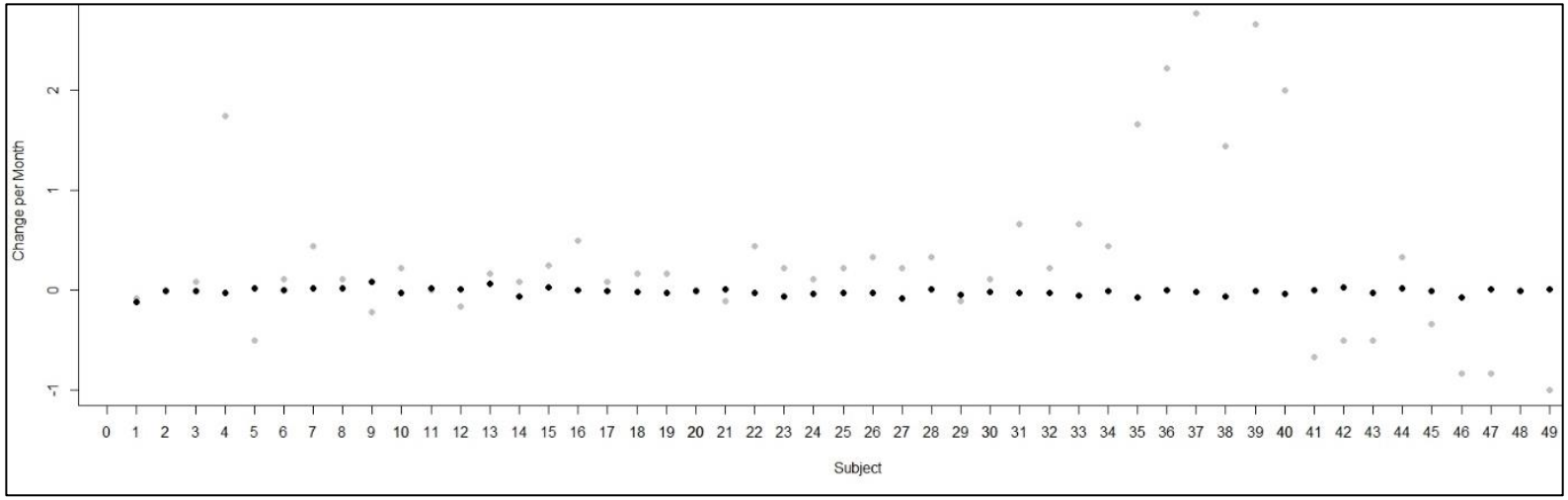

Fig. 10.: Telomere Dynamics and Weight Gain in NMRs

Although no significant relation between weight gain and telomere dynamics in NMRs were observed, the p-value (p=0.2) here indicates a possible trend. Telomere dynamics expressed as change in T/S-ratio per month (black dots) and change in weight (grey dots) in NMRs are shown in 49 animals.

4. Discussion

4.1 Molecular Sexing

A molecular sexing protocol has been successfully established for NMRs that can now be used for future studies. It can serve as a tool to either completely replace traditional, visual sexing techniques or to back up the results obtained by visual assessments.

Furthermore, it was found that the male to female sex ratio of NMRs in this study was 2.15:1. Whereas a male bias has been described before in NMRs (Jarvis et al. 1991), the occurring magnitude in our study population is high in comparison. In the colonies observed by Jarvis et al. (1991) male to female sex ratios in wild-caught animals were found to range between 1.12:1 and 1.40:1. However, male sex bias at birth is an often-observed phenomenon in many mammals – although, the underlying mechanisms are not well understood. Thus, it is conceivable that NMRs generally show a male sex ratio bias at birth, which subsequently equalizes until the animals reach adult age, due to a higher rate of male deaths during their developmental stages. This would be similar to the situation in humans, where a higher rate of male childhood mortality is thought to cause the displayed male sex ratio bias at birth (Gellatly 2019). Following this thought it would be a plausible scenario that factors leading to a higher male mortality in wild NMRs might be absent in captivity, resulting in an overall male sex bias.

Another theory - known as the local resource competition theory - suggests that natural selection might shift the equilibrium towards the dispersing sex (Silk and Brown 2000, Gellatry 2019). However, it is unclear if it is indeed only males that disperse in larger proportions in NMRs. While O'Riain et al. (1996) observed only male dispersers in a laboratory setting, Braude (2000) found that both females and males leave their nascent colonies to form new ones and that they do so in equal numbers in the wild.

Furthermore, in cooperative breeders the local resource enhancement theory would predict, that the most helpful sex would be favored and thus over-represented (Silk and Brown, 2008). Yet, while NMRs are a highly cooperative species, both sexes engage in helping behavior (Jarvis 1991).

Interestingly, Chau et al. (2018) analyzed the sex ratio of NMRs in 3 different zoo populations in the US (Zoo Atlanta, San Diego Zoo and Smithsonian National Zoological Park) and in contrast to the results presented here, found balanced numbers of each sex. Furthermore,

their dataset predominantly consisted of deceased juvenile NMRs – challenging the idea of a male sex ratio bias at birth (Chau et al. 2018).

However, sex allocations in mammals are known to vary within species (Ferrandiz-Rovira et al. 2016) and sex biases have been identified in many species and populations - including humans (Gellatly 2019), edible dormice (*Glis glis*, Ferrandiz-Rovira et al. 2017), common vampire bats (*Desmodus rotundus*, Delpietro et al. 2017) and golden-mantled ground squirrels (*Callospermophilus laterialis*, Wells and Vuren 2017). Thus, it seems possible that the observed male sex bias in this study depicts a population bias rather than a general one. Indeed, there are many probable reasons and corresponding theories for the occurrence of such imbalanced sex allocations. One would be the frequency dependent selection theory – which suggests when, for example, females become too abundant an adjustment takes place and more males emerge in future litters to achieve sex equilibrium. Also, the environmental conditions as well as the conditions of the parents determine the sex ratio of the offspring. Well-nourished mothers tend to give birth to more sons, since healthy sons are reproductively more successful than daughters (Gellatly 2019, Chau et al 2018). Also, testosterone levels at conception can influence the sex ratio of the litter and reproductive females with elevated circulating testosterone levels are more likely to give birth to male offspring (Gellatly 2019).

4.2 Establishing a qPCR Protocol to Measure Relative Telomere Length in NMRs

In an optimal qPCR assay the qPCR efficiency (Ct) should be close to 100%. In this study an efficiency (Ct) of 90% for telomere runs was achieved and 89% for non-variable copy number gene runs, respectively. Arguably, the efficiency could be further improved by introducing a few strategies into future protocols. For example, by optimizing DNA-extraction methods or by using multiple extractions (Eastwood et al. 2017). According to Olsen et al. (2012) also primer optimization strategies can lead to a better performance. Also, the use of different qPCR master mixes has been reported to influence qPCR efficiencies (Morinha et al. 2019).

4.3 Telomere Dynamics in NMRs

Significant changes in relative telomere length have not been detected between animals of different social status (queens vs. workers), or between the sexes (females vs. males) and only a trend might be deducible for weight gain in dependence of telomere dynamics. In NMRs

queens are often believed to outlive workers (Bens et al. 2018). But it is unclear whether this gap in life expectancy between animals of different social status is caused by individual aging patterns that underline the inherent animal's quality or due to lifestyle differences with workers engaging in riskier tasks within the colony. The findings of this study point to the latter, since the rates of telomere shortening here are similar between animals of different social status. Furthermore, every animal within an NMR colony possesses the potential to gain reproductive status once the hormonal suppression exercised by the current queen is removed (Ruby et al. 2018). However, this would still allow for different aging patterns between males and females, which is indeed an often-observed feature in mammals (Barrett and Richardson, 2011). Yet, no significant differences were found in telomere dynamics between male and female NMRs. These findings are in concordance with Ruby et al. (2018), who also reported a lack of demographic aging in NMRs. Furthermore, differences in telomere attrition between the sexes are often more pronounced in species with sexual size dimorphism and rarely seen in monomorphic species (Barrett and Richardson, 2011) – to which NMRs belong.

Only between weight change and change in relative telomere length a relationship is deducible (trend). Weight gain in NMRs occurs mainly at two points in their life - during their initial growth phase within the first few months of life and when they become reproductive members of the colony; either by replacing a former queen in their nascent colony or by forming a new colony (Jarvis 1991, Henry et al. 2006). The change in social status from worker to queen is accompanied by physical changes, including rapid lateral growth and weight gain. In the used dataset (49 animals from five colonies) five pups (age ≤ twelve months) were included, as well as one newly emerging queen. However, potentially pregnant queens were not excluded. Thus, weight gain that occurs during gestation cannot be ruled out and might have impacted the dataset. Growth is often accompanied by a rapid shortening of telomeres, that slows down once an animal reaches its adult size and weight (Monaghan and Ozanne 2018). In conclusion, no differentiation between weight change due to growth, change in social status or due to gestation was possible. Future studies should include these factors. However, the preliminary results obtained from this thesis might serve as an indicator for future studies. It might be interesting to see, whether there is a link between life trajectories and telomere dynamics in NMRs. Furthermore, there might be an observable shift in telomere dynamics when female NMRs become queens.

Additional limitations were present within this study. First, the age of the participating animals was unknown. Thus, only differences over a very short time period in relative telomere length

could be observed, regardless of the animal's age. Thus, making it impossible to directly compare animals of the same age. Furthermore, telomere dynamics in other species are known to be age-dependent and vary in accordance with the developmental stage (Monaghan and Ozanne 2018).

Second, the time restriction of this study only allows for a very narrow glimpse into the aging dynamics of NMRs. Giving the longevity of the study species of more than 30 years and the lack of cellular senescence until a very old age (Bens et al. 2018), major changes in telomere dynamics might not be captured within the time window of the study.

Furthermore, original telomere length is considered a marker of longevity. Additionally, it is known to differ between individuals. Thus, comparing the absolute telomere length in individuals of known age could provide further insight into telomere dynamics in NMRs.

5. Conclusion

5.1 Molecular Sexing

A molecular sexing protocol based on a multiplex PCR approach was successfully established. Subsequent sexing of 85 NMRs revealed a male sex ratio bias of 2.15:1. Since other studies do not support the existence of a general male sex ratio bias in NMRs, it is conceivable that the observed bias in this study depicts a population bias rather than a general one.

5.2 Establishing a qPCR Protocol to Measure Telomere Dynamics in NMRs

A qPCR protocol based on the Cawthon method (Cawthon 2002) was established to measure relative telomere length in NMRs. The assays qPCR efficiencies (Ct) were well within the range of what is generally regarded as good, which is close to 100% (90% for telomere runs and 89% for non-variable copy number gene runs, respectively). However, further improvements might be attainable by optimizing DNA extraction strategies, the use of different primers or trying different qPCR master mixes (Eastwood et al. 2017, Olsen et al. 2012, Morinha et al. 2019).

5.3 Telomere Dynamics in NMRs

No relationship between changes in relative telomere length per month and sex, social status (queen vs. worker) or weight change per month was detected. However, telomere dynamics in other species are known to be age-dependent and vary in accordance with the developmental stage (van Lieshout et al. 2019, Monaghan and Ozanne 2018). Unfortunately, in this study the age of the animals was unknown. Furthermore, the time restriction of the present thesis only allowed for a relatively short study period of one year. Giving the longevity of NMRs, which can live for more than 30 years and the lack of cellular senescence until a very old age (Bens et al. 2018), major changes in telomere dynamics might not be captured within the narrow time window of this study. Therefore, it might be well advised to elongate the time period of future studies that focus on telomere dynamics in NMRs and include study subjects of known age. Thus, enabling the detection of alterations in telomere length due to developmental changes and aging.

6. Abstract

The naked mole-rat (*Heterocephalus glaber*, henceforward abbreviated as NMR) is a mouse-sized rodent, that weighs on average only around 40 g per animal but exhibits a life-expectancy of more than 30 years (Blagosklonny 2013). Furthermore, reproductive females (queens) pose a contradiction to the disposable soma theory of aging, since they are often believed to be the oldest individuals within their colonies (Bens et al. 2018). Telomeres - a common feature found in all eukaryotic organisms - are proposed markers of aging (Bernadotte et al. 2016). With time telomeres gradually shorten and short telomeres are associated with higher mortality and decreased longevity (Haussmann and Mauck 2008). Nonetheless, telomeres can also elongate (e.g. Hoelzl et al. 2016). Given the behavioral and morphological differences between queens and other colony members, it is conceivable that reproductive females show a distinct pattern of telomere dynamics. Within this thesis, a qPCR-method (based on the Cawthon method (Cawthon 2002)) has been established to elucidate, whether there is a difference in telomere dynamics in naked mole-rats of different social status, sex and in dependence of the individuals' growth. To measure relative telomere length, 100 genomic DNA samples from 50 different animals, taken at two distinct timepoints were processed. Mean qPCR efficiencies (Ct) for telomere runs reached 90% and 89% for non-variable copy number gene runs, respectively. The inter-assay variation (mean coefficient of variation) for Ct values was 0.5% for telomere runs and 0.9% for non-variable copy number gene runs. The obtained relative telomere length-values of 49 animals were subsequently used to calculate a mixed linear model. Predictor variables were sex, social status (queen or worker) and growth (change in body weight per month). Changes in relative telomere length per month were unrelated to the animals' sex ($p=0.86$), social status ($p=0.61$) or growth ($p=0.2$). Furthermore, a molecular sexing approach based on multiplex PCR was established to facilitate the sex determination of naked mole-rats. To this end, DNA was extracted from buccal mucosa samples deriving from 85 animals and subsequently processed. Results revealed a male sex ratio bias of 2.15:1. Since other studies do not support the existence of a general male sex ratio bias in NMRs, it is likely that the observed bias depicts a population bias rather than a general one. The information gained and the protocols developed within this thesis can now be used as a take-off point for future studies.

7. Zusammenfassung

Nacktmulle (*Heterocephalus glaber*) sind Maus-große Nager mit einem durchschnittlichen Gewicht von nur 40 g pro Tier, aber einer erstaunlichen Lebenserwartung von mehr als 30 Jahren (Blagosklonny 2013). Zusätzlich werden fortpflanzungsfähige Weibchen (Königinnen) oftmals als die ältesten Tiere innerhalb einer Nacktmullkolonie betrachtet, wodurch diese einen Widerspruch zur “Disposable Soma Theory of Aging” bilden (Bens et al. 2018).

Telomerische Sequenzen an den Endstücken der DNA sind ein gemeinsames Merkmal aller eukaryontischen Organismen und werden gerne als molekulare Marker des Alterns herangezogen (Bernadotte et al. 2016). Telomere verkürzen im Laufe der Zeit schrittweise und kurze Telomere werden mit einer höheren Mortalität und einer eingeschränkten Langlebigkeit in Verbindung gebracht (Haussmann and Mauck, 2008). Betrachtet man die ethologischen und morphologischen Unterschiede zwischen Nacktmull-Königinnen und anderen Mitgliedern innerhalb der Kolonie (Jarvis 1991), so scheint es annehmbar, dass sich auch die Telomer-Dynamik fortpflanzungsfähiger Weibchen von der der anderen Kolonie-Mitglieder unterscheidet. Innerhalb dieser Masterarbeit wurde eine qPCR-Methode - basierend auf der Cawthon-Methode (Cawthon 2002) – etabliert. Mit ihrer Hilfe sollte geklärt werden, ob es einen Unterschied in der Telomer-Dynamik bei Nacktmullen abhängig vom sozialen Status, dem Geschlecht und / oder dem individuellen Wachstum gibt.

Um die relative Telomerlänge zu bestimmen, wurden 100 Proben genomischer DNA von 50 unterschiedlichen Nacktmullen herangezogen. Diese wurden an zwei getrennten Zeitpunkten genommen und miteinander verglichen. Die durchschnittliche qPCR-Effizienz (Ct) erreichte 90% für Telomer-Läufe und 89% für Läufe mit einem Non-Variable Copy-Number-Gen. Die Interassayvarianz (durchschnittlicher Variationskoeffizient) für Ct-Werte betrug 0,5% für Telomer-Läufe, beziehungsweise 0,9% für Non-Variable Copy-Number-Gen-Läufe. Die so erhaltenen Werte der relative Telomerlänge von 49 Tieren wurden schließlich verwendet, um ein linear gemischtes Modell zu berechnen. Als Prädiktorvariablen wurden Geschlecht, sozialer Status (Königin oder Arbeiter) und Wachstum (Körpergewichtsveränderung pro Monat) herangezogen. Die Ergebnisse zeigten, die Veränderungen der relative Telomerlänge pro Monat waren unabhängig vom Geschlecht der Tiere ($p=0.86$), ihrem sozialen Status ($p=0.61$) oder dem Wachstum ($p=0.2$).

Eine Methode zur molekularen Geschlechtsbestimmung basierend auf einer Multiplex-PCR wurde zusätzlich etabliert, um die Geschlechtsbestimmung bei Nacktmullen zu erleichtern. Zu

diesem Zweck wurde DNA von 85 verschiedenen Individuen aus deren bukkaler Mundschleimhaut extrahiert und verwertet. Die Ergebnisse zeigen ein Geschlechterverhältnis von 2,15:1 Männchen zu Weibchen. Andere Studien stützen dieses Ergebnis nicht (Chau et al. 2018), weswegen nicht von einem generellen, zu Gunsten der Männchen verzerrten Geschlechterverhältnis ausgegangen werden kann und ein Populations-Bias als Erklärung naheliegend scheint. Die innerhalb dieser Arbeit gewonnen Erkenntnisse und etablierten Protokolle können nun genutzt werden, um als Ausgangspunkt zukünftiger Studien zu dienen.

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9. List of Figures and Tables