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Effects of age on oxidative stress and locomotion in the pollinator, *Megachile rotundata*

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ABSTRACT

Despite numerous aging studies, the relationship between oxidative stress, aging, and decline in functions such as locomotion is still debated. Insects offer a promising model for analyzing the relationship between oxidative stress and aging, because they exhibit vast differences in lifespan that may be affected by the environment, social factors, levels of activity, and aging interventions. In this study, we explore the effects of aging on oxidative stress and locomotion using the pollinator, *Megachile rotundata*, a species that is very mobile and active in the adult stage. Across the adult lifespan *of M. rotundata*, we assessed changes in walking, flight, oxidative damage, and antioxidant defenses. Our results suggest that *M. rotundata* experience age-related declines in flight, but not walking. Additionally, we found that oxidative damage and antioxidant capacity initially increase with age and physical activity, but then levels are maintained. Overall, these data show that *M. rotundata*, like some other organisms, may not perfectly follow the free radical theory of aging.

1. Introduction

Aging is a progressive deterioration of physiological functions that impairs an organism's ability to maintain homeostasis, ultimately leading to death (Perridon et al., 2016). As a result of aging, nearly all organisms experience functional senescence in reproduction, cognition, and locomotion. Locomotor tasks such as walking and flight have been shown to decline with age, and senescence is thought to arise from damage to the nervous and muscular systems (Mecocci et al. 1999; Martin and Grotewiel, 2006). Both the nervous and muscular systems are prone to damage due to their high rates of metabolism and production of reactive oxygen species (ROS; Martin and Grotewiel, 2006; Halliwell and Gutteridge, 2015). The free radical theory of aging postulates that oxidative stress, the imbalance between the production of ROS and the body's antioxidant defense system, leads to age-dependent accumulation of damage in macromolecules, which progressively deteriorates physiological functions, leads to declines in locomotion, and shortens lifespans (reviewed in Martin and Grotewiel, 2006; Monaghan et al., 2009; Metcalfe and Alonso-Alvarez, 2010).

Due to their instability, ROS, are highly reactive molecules that can damage lipids, proteins, and DNA. Oxidative damage to lipids can lead to the inactivation of cellular proteins, impairment of membrane fluidity and elasticity, and even apoptosis (Wickens, 2001; Birben et al., 2012). Protein oxidation can induce carbonylation, cross-linkage, fragmentation, and oxidation of amino acid residues of proteins (Wickens, 2001; Birben et al., 2012). Oxidative damage to DNA, can cause degradation of bases, deletions, mutations, and single and double breaks (Wickens, 2001; Birben et al., 2012).

To counterbalance the effects of ROS, organisms use an array of antioxidants to reduce the production of oxidative damage (reviewed by Monaghan et al., 2009; Tan et al., 2018). Despite the ROS protection provided by these antioxidants, oxidative damage still occurs even under normal physiological conditions (Sohal et al., 1994). Furthermore, it is thought that effectiveness of certain antioxidant defense decreases with age (Sohal et al., 1984; Bunker, 1992; Tan et al., 2018; Maldonado et al., 2023). Together, the accumulation of oxidative damage, reduced antioxidant defenses, and reduced repair for oxidative damage with age are thought to be the primary cause of aging and age-related declines in locomotion.

Although there have been numerous studies supporting the free radical theory of aging, there has also been increasing evidence that the theory doesn't perfectly fit all organisms (reviewed in Buffenstein et al., 2008; Pérez et al., 2009; Speakman and Selman, 2011; Stuart et al., 2014; Speakman et al., 2015). For example, in a study looking at the

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relationship between oxidative damage and antioxidant capacity in aging sea urchins, oxidative damage did not accumulate, and antioxidant activity was maintained with age, suggesting that sea urchins experience a negligible senescence (Du et al., 2013). Similarly, aging inconsistencies have been reported in insects, with accumulation of oxidative damage and changes in antioxidant defense differing greatly between species (Fleming et al., 1992; Sohal et al., 1995; Margotta et al., 2018; Kramer et al., 2021) and within species, such as castes in social insects (Corona et al., 2005; Seehuus et al., 2006; Schneider et al., 2011; Elsner et al., 2018; Monroy Kuhn et al., 2019; Quque et al., 2019; Kramer et al., 2021). Due to the discrepancies we see across and within species, more aging research using non-model insects is needed to provide a more complete understanding of the aging process.

Insects are essential for understanding aging theories, including the free radical theory of aging, because they exhibit vast differences in lifespan and rates of functional senescence. It is thought that insects exhibit these differences due to environmental and social factors, levels of activity (prolonged flight), and aging intervention, such as diapause, a state of programmed developmental arrest coupled with suppressed metabolic activity (reviewed in Guo et al., 2020). With both agricultural relevance and life-history traits (including solitary behavior, high-levels of activity, and diapause) that will provide insight into aging, *Megachile rotundata* presents a system to test age-related hypotheses. *Megachile rotundata* experience high levels of activity because they live independently without any workers to share duties. Female *M. rotundata* solely provide for their offspring, constructing nests for about 7.5 h a day (Klostermeyer and Gerber, 1969), most of which is spent flying with up to 130 foraging trips being made in single day (Unpublished data, Pithan). Additionally, *M. rotundata* is a facultative diapausing bee; some individuals go through direct development, but others overwinter by entering diapause (Pitts-Singer and Cane, 2011).

In this study, we establish a connection between *M. rotundata* age, oxidative stress, and locomotion senescence. To examine the effects of aging we assessed changes in walking and flight, as well as levels of lipid peroxidation, protein carbonylation, and antioxidant capacity in whole bees. We hypothesize that both walking and flight would decline with age due to the accumulation of oxidative damage caused by the imbalance between antioxidant capacity and ROS production.

2. Methods

2.1. Rearing and maintenance of M. rotundata

In 2021 and 2022, overwintering *M. rotundata* prepupae were obtained from JWM Leafcutter Inc. (Nampa, Idaho) and stored in an incubator (Conviron, Winnipeg, Manitoba) at 6 °C to maintain post-diapause quiescence until the beginning of the study, as previously done (Rinehart et al., 2016). At the start of the study, prepupae were removed from storage and placed into a 29 °C incubator (Precision Scientific, Buffalo, New York) to initiate metamorphic development. Newly emerged *M. rotundata* (<24 h) were placed into housing containers (12 × 12 × 3 cm) with 5 males and 5 females. *M. rotundata* emerge sexually mature and mating behavior was observed in the housing containers. The housing containers were then stored in an incubator (Precision Scientific, Buffalo, New York), with a photoperiod of 17:7 (L:D) cycles. The temperature was set to 29 °C during the light cycle and 25 °C during the dark cycle. Additionally, the humidity in the incubator was maintained at 70 %. Throughout the study, *M. rotundata* were given *ad libitum* access to Prosweet (Mann Lake Ltd., Hackensack, Minnesota) diluted to 50 % with deionized water that was replaced three times a week to prevent the spread of food-borne pathogens. Individuals were used only once and measured at one of four time points after adult emergence: day 0, 7, 14, or 21. The following experiments were replicated until a sufficient number of observations were made (Supplementary Table S1).

2.2. Lifespan

Following placement in the housing container, a subset of *M. rotundata* (n: females = 163, males = 169) were monitored daily until all individuals died of natural senescence under the lab conditions mentioned above. Individuals were recorded as dead if they failed to respond to mechanical stimulation with a cotton swab.

2.3. Walking performance

To measure age-dependent changes in walking performance, *M. rotundata* (n: females = 287, males = 153) were placed into a 32-channel locomotion activity monitor (LAM; Trikinetics, Waltham, Massachusetts). Individuals of known age and sex (0, 7, 14, and 21 days) were individually separated into 7 ml tubes. Each tube was placed into one of the channels in LAM within an incubator. Prior to collecting data, individuals within the tubes were allowed to acclimate for 15 min at 29 °C. Similar to housing conditions, the walk activity incubator had a 17:7 (L:D) photoperiod with temperature at 29 °C during the light cycle and 25 °C during the dark cycle. The number of movements was counted every minute for 24 h.

2.4. Flight ability

Age-dependent changes in flight ability in *M. rotundata* (n: females = 215, males = 153) were assessed using a cylinder drop test assay (Banerjee et al. 2004; Earls et al., 2021). To approximate optimal flight conditions, the drop test was performed indoors with an ambient temperature of 29 °C and a light intensity of 18,000 lx. Prior to the drop test, *M. rotundata* (0, 7, 14, and 21 days) were placed individually into cups (4 × 4.5 cm, ID × H). Individuals were dropped into an acrylic cylinder (23 × 89 cm, ID × H) and considered successful at the drop test if they were able to slow their descent or generate upward lift. Individuals were scored as being able to slow their descent if they displayed longitudinal hovering while gliding and flapping wings. Each individual was given three attempts to succeed at the drop test; those that fell directly through after the third attempt were scored as non-fliers. Individuals were given three attempts to learn the assay.

2.5. Lipid peroxidation

Lipid peroxidation levels were estimated using a TBARS assay that measured malondialdehyde (MDA; López-Martínez and Hahn, 2012). Malondialdehyde is used as an indirect biomarker of oxidative stress in a variety of organisms, including insects (Magwere et al., 2006; Dubovskiy et al., 2008; Margotta et al., 2018). Females and males (n: females = 74, males = 73), ages 0, 7, 14, and 21 days, were homogenized individually in 350 μl RIPA buffer with EDTA (Fisher Scientific, Pittsburg, Pennsylvania) using a Bead Blaster 24 (Benchmark Scientific, Sayreville, New Jersey). An aliquot of 100 μl homogenate was used to quantify protein content for standardization of MDA levels using an Epoch Microplate Spectrophotometer (BioTek, Winooski, Vermont) with a set absorbance reading of 280 nm, while the remaining homogenate was treated with 200 μl of 10 % trichloroacetic acid (TCA) to precipitate proteins. The aliquot treated with TCA was then combined with a 0.67 % thiobarbituric acid solution (TBA), and 150 μl of the sample was pipetted in triplicate into a 96-well plate, and absorbance was read at 532 nm. Quantification of MDA is presented as nmol/ng of protein.

2.6. Protein carbonylation

Protein carbonylation was measured using a protocol modified for insects (López-Martínez and Hahn, 2012). Protein carbonyls are composed of groups, such as aldehydes or ketones, added to protein chains as consequence of oxidation of proteins (Dalle-Donne et al., 2003). Protein carbonyls are highly stable and easy to detect making

them ideal for protein damage quantification (Dalle-Donne et al., 2003). Females and males (n: females = 32, males = 32), ages 0, 7, 14, and 21 days, were individually homogenized as described above and treated with 500 µl of 2,4-dinitrophenylhydrazine (DNPH; Fisher Scientific, Pittsburgh, Pennsylvania) to extract carbonyls. Sample carbonyls were then precipitated with TCA. The resulting protein precipitate was diluted in 600 µl of 6 M guanidine hydrochloride (GuHCl). Using 200 µl of the sample with GuHcl the absorbances of the samples were read in triplicate at 370 nm using the Epoch Microplate Spectrophotometer. The data are presented as nmol/mg of protein.

2.7. Antioxidant capacity

To assess changes in antioxidants with age, total antioxidant capacity was measured using Trolox equivalent antioxidant capacity (TEAC) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid; ABTS). A TEAC assay was used because it measures the cumulative action of all water-soluble antioxidants and thus, prevents overlooking unknown antioxidants and the potential synergistic interactions between antioxidants (Ghiselli et al., 2000). Individuals (n: females = 68, males = 68), ages 0, 7, 14, and 21 days, were homogenized in 700 µl phosphate buffered saline (PBS) using Bead Blaster 24 and separated into two aliquots. One aliquot was used for quantifying protein content using an Epoch Microplate Spectrophotometer set at 280 nm. The second aliquot of the homogenized sample was diluted to a concentration of 2 mg protein/ml and combined with a solution of 7 mmol ABTS and 2.45 mmol potassium persulfate (Sigma-Aldrich, Burlington, Massachusetts). The ABTS solution was allowed to react for 24 h in darkness at 25 °C before adding it to samples. Samples with ABTS solution were then loaded in triplicate into 96-well plates and measured at 734 nm. This last step took place in under 10 min to prevent the reaction from being unstable. Quantification of TEAC is presented as µmol/mg of protein.

2.8. Statistical analysis

Statistical analyses were performed in JMP (version 17.0.0, SAS Institute, Cary, North Carolina). Normality was tested via Shapiro-Wilk test, while variance was assessed using a Levene's test. Differences in lifespan were determined using a proportional hazard and presented as percent survivorship. Among age groups, success at the drop test was analyzed using a binominal logistic regression, and the number of attempts needed to succeed was analyzed using an ordinal logistic regression. Two way-ANOVAs with Tukey's HSD *post hoc* tests were used to compare the means of protein carbonylation, and antioxidant capacity. Due to non-normal distribution and unequal variance, walking activity and lipid peroxidation were analyzed using a Kruskal-Wallis with Wilcoxon method for nonparametric comparisons between age groups. For every statistical test, sex, age, and the interaction between the factors were included in the models. Replication was initially included as a factor in model, but was removed due to non-significance. *P*-values less than 0.05 were considered statistically significant. Data are represented as means ± 95 % confidence intervals throughout.

3. Results

3.1. Lifespan

The lifespan of male and female *M. rotundata* under lab conditions significantly differed (Table 1; Fig. 1), with females living longer. Of the 163 females observed, the average life expectancy was 17.40 ± 0.84 days with the lifespans ranging from 5 to 31 days. The average life expectancy of the 169 males observed was 13.33 ± 0.68 days with the lifespans ranging from 3 to 24 days.

Table 1
Statical results, including test statistic (X^2 or F), degrees of freedom (d.f.), and p-value, for age and sex effects on lifespan, walking performance, flight ability, oxidative damage (lipid peroxidation and protein carbonylation), and total antioxidant capacity in *M. rotundata*.

Experiment (statistical test)	X^2	F	d.f.	P-value
Lifespan (Proportional hazard)				
Sex	40.285	–	1, 323	<0.0001
Walking performance (Kruskal-Wallis)				
Sex	30.405	–	1, 439	<0.0001
Female age	46.332	–	3, 286	<0.0001
Male age	31.21	–	3, 152	<0.0001
Flight ability: Success at drop test (Binominal logistic regression)				
Full model	7.352	–	3, 391	0.062
Age	128.452	–	3, 391	<0.0001
Sex	1.099	–	1, 391	0.294
Flight ability: Attempts needed to succeed (Ordinal logistic regression)				
Full model	0.878	–	3, 391	0.645
Age	19.07	–	3, 391	<0.0001
Sex	0.611	–	1, 391	0.435
Lipid peroxidation (Kruskal-Wallis)				
Age	63.453	–	3, 146	<0.0001
Sex	2.698	–	1, 146	0.101
Protein carbonylation (ANOVA)				
Full model	–	0.72	3, 63	0.544
Age	–	4.101	3, 63	0.011
Sex	–	1.168	1, 63	0.284
Total antioxidant capacity (ANOVA)				
Full model	–	1.214	3, 135	0.307
Age	–	40.953	3, 135	<0.0001
Sex	–	2.862	1, 135	0.093

3.2. Walking performance

M. rotundata walking activity was significantly affected by age and sex with females having a higher level of walking activity compared to males (Table 1; Fig. 2). When the bees first emerged, females and males had an average walking activity of 666.06 ± 80.31 and 473.13 ± 70.47 movements/day, respectively. By day 7, there was a two-fold increase in walking activity for both males and females. Following day 7, walking activity was maintained for males with no significant changes among days 7, 14, and 21. There was no significant difference in walking activity for females when comparing day 7 and 14. However, there was significant decrease (15 % compared to day 14) in female walking activity at day 21.

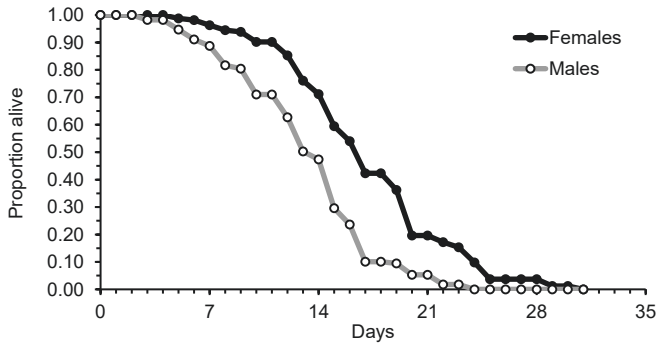

Fig. 1. Lifespan of male and female *M. rotundata* under laboratory conditions (n = 169 males and 163 females. Lifespan of male and females significantly differed.

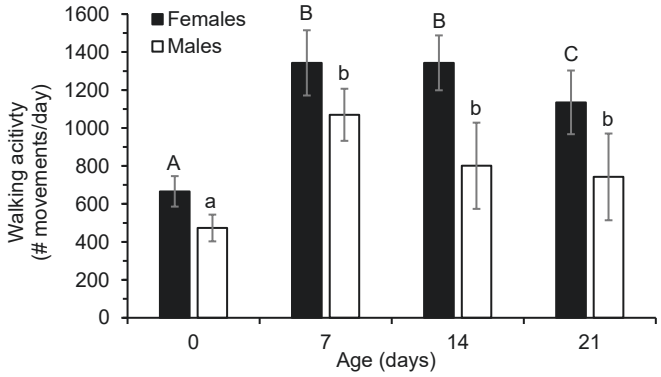

Fig. 2. Average daily walking activity at different ages (n = 20–90 per bar). Error bars represent 95 % confidence intervals. Males and females did differ. Ages with different letters were significantly different ($p < 0.05$), females (uppercase) and males (lowercase).

3.3. Flight ability

Age, but not sex, significantly affected the number of individuals that succeeded at the drop test and the number of attempts needed to succeed (Table 1; Fig. 3). When the adults first emerged, nearly 100 % were able to fly. The first decline in flight performance occurred on day 14 with the number of successful bees decreasing by 27 %. By day 21, only 32 % of the bees were able to succeed at the drop test.

Of the individuals that succeeded at the drop test, the majority of day 0 bees (93 %) were able to fly on the first attempt. By day 14, there was

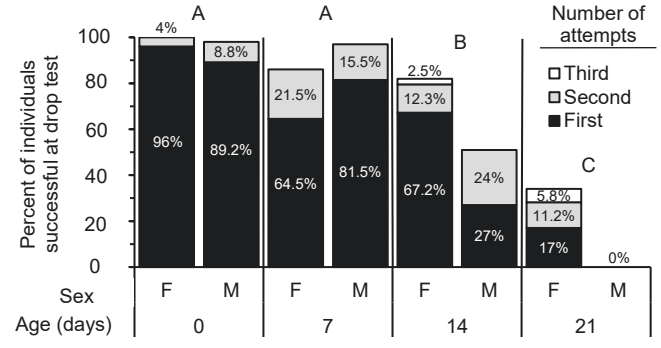

Fig. 3. Percent and number of attempts needed to generate upward lift or slow their decent during drop test (n = 11–64 per bar). Percentages presented in figure are out of the total number of individuals used in the drop test. Males and females did not differ. Ages with different letters were significantly different ($p < 0.05$).

significant increase in the number of attempts needed to succeed at the drop test with some individuals (3 %) needing a third attempt and increase in the number of individuals (13 % increase from day 0) needing second attempt.). By day 21, there was a continued shift in the number of attempts needed, with only 50 % of fliers being able to succeed on the first attempt and an increase to 17 % needing a third attempt.

3.4. Lipid peroxidation

There was a significant effect of age, but not sex on lipid peroxidation (Table 1; Fig. 4A). When the bees first emerged, levels of lipid peroxidation were relatively low, with *M. rotundata* having 0.62 ± 0.15 nmol/mg of protein. By day 7, there was over 3 times more lipid peroxidation when compared to recently emerged bees. Following day 7, lipid peroxidation levels were maintained until day 21 where there was a significant decrease (8 %) when compared to day 14.

3.5. Protein carbonylation

There was a significant effect of age, but not sex on protein carbonylation levels (Table 1; Fig. 4B). As shown in Fig. 4B, the protein carbonyl levels in recently emerged adults were statistically similar to 7 and 14-day old individuals. On day 21, there was a significant increase (32.9 % compared to recently emerged) in carbonylated proteins with *M. rotundata* on average having 93.056 ± 8.40 nmol/mg of protein.

3.6. Antioxidant capacity

Total antioxidant capacity was affected by age, but not sex (Table 1; Fig. 5). Similarly, to lipid and protein oxidative damage, the lowest level

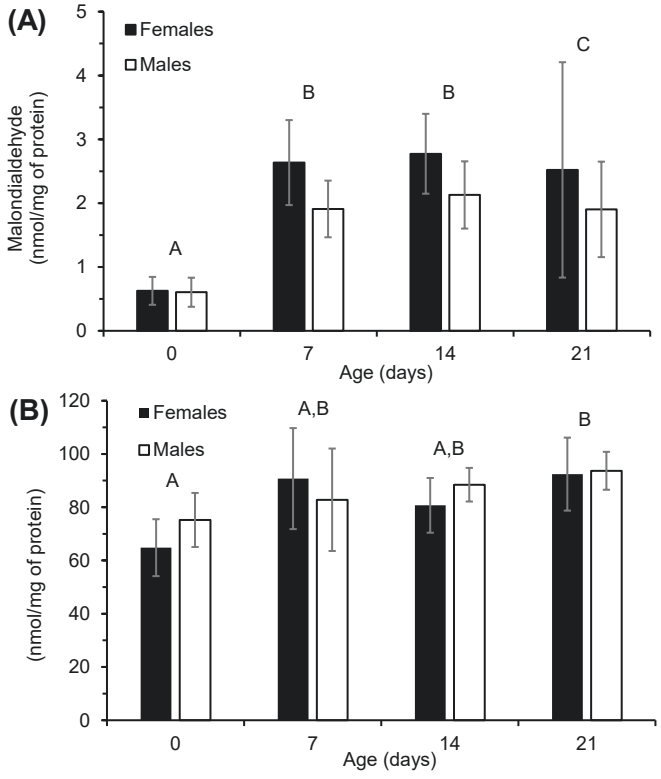

Fig. 4. Oxidative damage at different ages. (A) Average levels of lipid oxidative damage (n = 18–20 per bar). (B) Average levels of protein oxidative damage (n = 8 per bar). Error bars represent 95 % confidence intervals. Males and females did not differ. Ages with different letters were significantly different ($p < 0.05$).

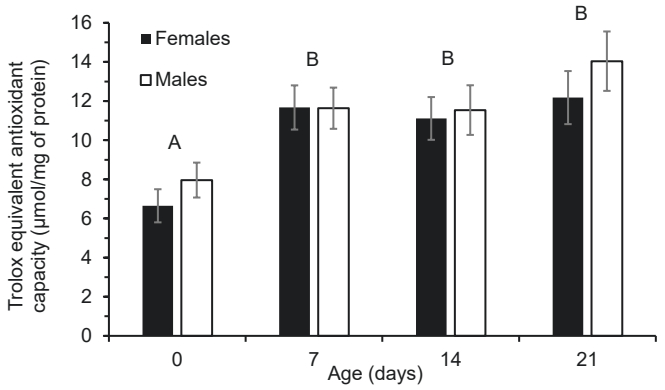

Fig. 5. Average total antioxidant levels at different ages (n = 16 per bar). Error bars represent 95 % confidence intervals. Males and females did not differ. Ages with different letters were significantly different ($p < 0.05$).

of total antioxidant capacity occurred when the bees first emerged. Recently emerged *M. rotundata* had a total antioxidant capacity of 7.31 ± 0.63 µmol/mg of protein. By day 7, antioxidant activity increased by 60 % and was maintained with no significant differences among days 7, 14, and 21.

4. Discussion

The free radical theory of aging postulates that an imbalance in oxidative damage and antioxidant defense leads to declines in physiological function and ultimately death (reviewed in Martin and Grotewiel, 2006; Monaghan et al., 2009; Metcalfe and Alonso-Alvarez, 2010). Although results have provided support for this theory since its introduction in the 1950′s (Harman, 1956), several recent studies have shown inconsistencies and contradictions to its predictions (reviewed in Buffenstein et al., 2008; Pérez et al., 2009; Speakman and Selman, 2011; Speakman et al., 2015). In the present study, *Megachile rotundata* did not behave as predicted by the free radical theory of aging. We establish that while flight ability is age-dependent, walking performance did not decline with age. We also found that although oxidative damage was affected by age, it increased only once after day 7, thus not performing as predicted by the free radical theory of aging. Additionally, we found no significant decline in antioxidant capacity with age, which may explain why oxidative damage did not increase as expected with age. Alternatively, this initial increase in oxidative damage and antioxidant capacity may be linked to changes in physical activity early on in life.

Our study shows age-dependent declines in flight ability with both a decrease in the number of individuals successful at flight and an increase in those needing more attempts with age. These age-dependent changes in flight ability of *M. rotundata* are comparable to declines in other species of insects. In *Aedes aegypti,* flight performance deteriorated with the distance flown decreasing by 42 % at the start of the third week and a continued decline into the fourth (Rowley and Graham, 1968). Similarly, several studies have shown that *Drosophila* experience declines in wing beat frequency (Williams et al., 1943; Miller et al.,2008; Lane et al., 2014) and ability to generate upward lift (Miller et al., 2008; Lane et al., 2014) with age. In honey bees (*Apis mellifera*), the flight capacity of foraging bees decreased with age and was likely due to the accumulation of oxidative damage (caused by superoxide and hydrogen peroxide) in flight muscles (Margotta et al., 2018). Similarly, oxidative stress and mechanical wing wear caused age-related declines in maximal kinematic and flight capacity in old foragers (Vance et al. 2009). The observed age-related declines in flight ability is likely to impact reproduction in *M. rotundata*. Female *M. rotundata* solely provide for their offspring, requiring them to make frequent, metabolically demanding flights to construct and provision brood cells for their young. This intense level of activity, coupled with age-related declines in flight ability, is expected to reduce reproductive output and offspring quality with parent age; however, this needs further study.

Similar to flight ability, several species of insects have displayed age-related declines in walking performance, including assassin bugs (Matsumura et al., 2021), cockroaches (Ridgel et al., 2003), flies (Cook-Wiens and Grotewiel, 2002; Gargano et al., 2005; Carey et al., 2006; Martinez et al., 2007), and ladybird beetles (Dixon and Agarwala 2002). However, contrary to our hypothesis and other aging studies, *M. rotundata*, within our time scale, did not experience age-related declines in walking activity, but an increase from when they emerged to the first week. The increase in walking activity may be due to increased use of the leg muscles. Post-eclosion muscle growth has been documented in thoracic muscles in a variety of insects, including ants (Muscedere et al., 2011; Matte et al., 2024), fruit flies (Chaturvedi et al., 2019), and hawk moths (Wone et al., 2018). It is possible that this also happens in leg muscles. The discrepancy in rates of locomotion senescence between flight ability and walking activity may be due to different metabolic demands. Flight in insects is costly, with flight metabolic rates being higher than metabolic rates while running (reviewed in Harrison and Roberts, 2000). The increase in metabolic demand during flight causes a 30–100 fold increase in oxygen consumption (Davis and Fraenkel, 1940; Tribe, 1966), which in turn elevates the production of ROS (Hulbert et al., 2007; Margotta et al., 2018) and is thought to damage flight muscles (Vance et al., 2009).

Prior studies in both insects and in mammals have shown age-dependent increases in protein carbonyl groups and lipid peroxides (Sohal et al., 1985; Sohal et al., 1993; Mecocci et al. 1999; Matsugo et al., 2000; Kramer et al., 2021). However, we did not find that. Lipid damage plateaued 7 days after emergence. Protein damage increased more gradually as expected (Levin et al., 2017). This mitigation of oxidative damage (lipid peroxidation and protein carbonylation) is likely due to the observed upregulation of antioxidant capacity. With age, there was a parallel increase in walking activity, oxidative damage and total antioxidant capacity. Similar to this study, Williams et al. (2008) found that when young honey bees first experience intense physical activity (foraging flight) there was an increase in both oxidative damage and antioxidant capacity. In response to the increase in locomotion and oxidative damage, it is likely that cells and tissues upregulated antioxidant levels to help prevent oxidative damage, thus mitigating the ongoing damage caused by ROS. The lack of increase in oxidative damage in later stages of life is likely because of the strong decline in flight ability, the main source of oxidative stress in animals with flight. Similar to this study a dissociation between aging and oxidative stress has been documented in honey bee foragers with no age-related decreases in total antioxidant capacity (Williams et al. 2008) or increases in protein carbonylation (Seehuus et al., 2006; Williams et al., 2008) and MDA (Margotta et al. 2018) within the brain. It is important to note that Margotta et al. (2018) did find discrepancies (oxidative damage and antioxidant levels) between the brain and flight muscle. In the present study, tissue specificity was not explored. Overall, this study coupled with other studies (Cook-Wiens and Grotewiel, 2002; Seehuus et al., 2006; Williams et al., 2008; Schneider et al., 2011; Margotta et al. 2018), may suggest that there is an additional mechanism (other than oxidative stress) for age-related mortality in insects.

Sex differences in oxidative stress in insects have been linked to various factors including differences in physiology, physical activity, and reproductive strategies (Ballard et al., 2007; Archer et al., 2013; Niveditha et al., 2017). However, in this study we found that there was no difference in oxidative damage and total antioxidant capacity between the sexes. This was surprising given that *M. rotundata* females exhibited longer lifespans, higher walking activity, and delayed flight senescence when compared to males. The sex differences in physical activity and lifespan are likely due to differences in biological roles and evolutionary pressures. Parental care in *M. rotundata* is performed by females that build the nests, provision and protect her offspring, while the male makes no contribution post-insemination (Pitts-Singer and

Cane 2011). Additionally, it has been found that male *M. rotundata* harass females leading to fewer and longer foraging trips resulting in fewer offspring (Rossi et al., 2010). While this may explain the difference in lifespan and physical activity, it does not explain the lack of difference in oxidative stress, a fundamental component of the free radical theory of aging. It is generally believed that females (in humans, mice, rats, and flies) experience less oxidative stress due to lower ROS production and upregulation of antioxidants, however, the results have been arguable, varying based on the cell type or tissue being investigated (reviewed in de Toda et al., 2023). It is possible that by us using whole body individuals, we were unable to detect differences in oxidative damage and total antioxidant capacity between sexes.

5. Conclusion

Using the agriculturally valuable pollinator, *M. rotundata*, this study provides new information on the relationship between aging, oxidative stress, and locomotion senescence. In this comprehensive study, we found that flight ability declines with age in females, and we suggest that it could lead to a decline in quality and number of offsprings with age. Additionally, we found a link between activity and oxidative damage). However, within our time scale, the lack of continued accumulation of oxidative damage and relatively steady antioxidant capacity in *M. rotundata* do not perfectly fit predictions of the free radical theory of aging likely because of the strong decline in flight performance, the main source of oxidative stress in flying animals. There is a clear correlation between the increases in oxidative damage, antioxidant capacity, and the increase in walking activity due to post-emergence muscle development. Overall, this study supports a call for increased understanding of the factors that affect performance and aging in all organisms.

CRediT authorship contribution statement

Jacob B. Pithan: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joseph P. Rinehart:** Supervision, Resources, Project administration, Funding acquisition. **Kendra J. Greenlee:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Giancarlo López-Martínez:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kendra Greenlee reports financial support was provided by USDA Agricultural Research Service. Kendra Greenlee reports financial support was provided by National Science Foundation. Giancarlo Lopez-Martinez reports financial support was provided by National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2024.104666.

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