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Honey bee (*Apis mellifera*) drones survive oxidative stress due to increased tolerance instead of avoidance or repair of oxidative damage

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ABSTRACT

Oxidative stress can lead to premature aging symptoms and cause acute mortality at higher doses in a range of organisms. Oxidative stress resistance and longevity are mechanistically and phenotypically linked; considerable variation in oxidative stress resistance exists among and within species and typically covaries with life expectancy. However, it is unclear whether stress-resistant, long-lived individuals avoid, repair, or tolerate molecular damage to survive longer than others. The honey bee (*Apis mellifera* L.) is an emerging model system that is well-suited to address this question. Furthermore, this species is the most economically important pollinator, whose health may be compromised by pesticide exposure, including oxidative stressors. Here, we develop a protocol for inducing oxidative stress in honey bee males (drones) via Paraquat injection. After injection, individuals from different colony sources were kept in common social conditions to monitor their survival compared to saline-injected controls. Oxidative stress was measured in susceptible and resistant individuals. Paraquat drastically reduced survival but individuals varied in their resistance to treatment within and among colony sources. Longer-lived individuals exhibited higher levels of lipid peroxidation than individuals dying early. In contrast, the level of protein carbonylation was not significantly different between the two groups. This first study of oxidative stress in male honey bees suggests that survival of an acute oxidative stressor is due to tolerance, not prevention or repair, of oxidative damage to lipids. It also demonstrates colony differences in oxidative stress resistance that might be useful for breeding stress-resistant honey bees.

1. Introduction

Molecular damage due to oxidative stress may be one of the primary causes of organismal aging (Finkel and Holbrook, 2000; Harman, 1956). Oxidative stress occurs when reactive oxygen species (ROS) are accumulating in a living system faster than they can be detoxified. Oxidative stress has been linked empirically to aging (Finkel and Holbrook, 2000) and age-related diseases such as diabetes, cancer, cardiovascular diseases, Parkinson disease, and Alzheimer disease (Beckman and Ames, 1998; Harman, 2006; Markesbery, 1997; Pandey et al., 2010). For example, selection for stress resistance results in long-lived flies (Rose et al., 1992), experimental up-regulation of enzymes that defend against ROS in *Drosophila* increases lifespan (Arking et al., 2000), and elevated concentrations of antioxidants contribute to longevity and longer life span (Radyuk et al., 2010; Svensson and Larsson, 2007).

ROS include (but are not limited to) peroxyl radicals, hydroxyl radicals, hydrogen peroxides, and superoxide anions (Farooqui, 2012) and may derive from endogenous and exogenous sources (Monaghan et al., 2009). ROS levels that exceed the capacity of cellular antioxidant defenses, such as detoxifying enzymes and radical scavenging molecules, cause lipid peroxidation of cell membranes, modification of proteins, DNA mutations or fragmentation, and potential cell death (Hughes and Reynolds, 2005). Oxidative stress causes dramatic changes in gene expression and cellular functions (Li et al., 2008; Zou et al., 2000). Individual cell components can be affected differently by oxidative stress, but surprisingly little is known on the overall relation between different classes of oxidative damage (Sohal, 2002).

Considerable natural variation in ROS susceptibility and longevity exists among and within species. The relatively long life of birds

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compared to similarly-sized mammals has been explained by lower ROS production or biomolecules that are less susceptible to ROS damage (Costantini, 2008). Overexpression of antioxidant enzymes decrease oxidative damage and increase life expectancy in *Drosophila melanogaster* (Sohal and Weindruch, 1996). However, the mechanisms of natural intraspecific variation have not been sufficiently elucidated. Surviving oxidative stress could be due to prevention, repair, or tolerance of molecular damage. This distinction has not been sufficiently investigated although exceptional longevity in humans has been characterized in terms of these three categories (Evert et al., 2003).

Although oxidative stress is studied in a variety of insects (Holmstrup et al., 2011; Kodrík et al., 2007; Krishnan et al., 2007), studies that link oxidative stress with aging and survival are largely restricted to *D. melanogaster* and a few other dipterans (Sohal et al., 1995). However, a broad comparative data basis, including relatively long-lived species, is needed for a comprehensive understanding of the links between oxidative stress and aging (Costantini, 2008). Social insects in the order Hymenoptera are exceptionally long-lived and display a very high degree of intraspecific plasticity in life expectancy (Carey, 2001; Rueppell et al., 2004). The best studied social insect is the honey bee, *Apis mellifera* (L). This species is a long-standing research model with a completely annotated genome (Weinstock et al., 2006) and it is the most important pollinator in a variety of natural and agricultural ecosystems (Calderone, 2012). Thus, the continued decline of honey bee health is particularly concerning. While no single cause for honey bee decline has been identified, a combination of factors may be responsible, including various pesticides (Goulson et al., 2015).

In honey bee females, the reproductive protein vitellogenin plays an important role as an antioxidant that may explain the aging plasticity between female castes and individual variation (Amdam et al., 2009; Amdam and Omholt, 2002; Corona et al., 2007; Seehuus et al., 2006). Specifically, the hemolymph titer of vitellogenin is directly linked to survival of acute oxidative stress (Seehuus et al., 2006) and classic antioxidant defenses may be less important for explaining differences in life expectancy between honey bee castes (Corona et al., 2005). In contrast, male honey bees (= drones) have much lower levels of vitellogenin (Piulachs et al., 2003) and may be more susceptible to oxidative stress due to their haploidy (Stürup et al., 2013). However, drones display mortality dynamics under natural conditions that are similar to workers (Rueppell et al., 2005).

Oxidative stress in honey bee drones has not been studied even though they are essential to the honey bee life cycle. Drones mature inside the colony and most initiate flights to mating arenas, called drone congregation areas, when they are 8–10 days old (Rueppell et al., 2005). Staying airborne in the drone congregation areas, the drones wait for mating partners to arrive and pursue them for a chance of copulation. Thus, drones' flight muscles are critical to secure mating opportunities and strong selective pressure exists for their protection and maintenance during the mating flight period that can last over 30 days (Rueppell et al., 2005).

Oxidative stress may be caused by endogenous and factors (Finkel and Holbrook, 2000). Environmental stressors that generate oxidative stress and can contribute to human disease include pesticides and other xenobiotics (Sone et al., 2010). These toxins either generate ROS directly or indirectly as a by-product of their cellular detoxification or compensatory energy generation (Lukaszewicz-Hussain, 2010). Exposure of colonies to pesticides results in significant physiological stress in honey bee workers and queens (Henry et al., 2012; Maini et al., 2010). However, only a few studies have investigated the molecular mechanisms of pesticide stress in drone honey bees and possible mechanisms to survive pesticide exposure (Johnson et al., 2013; Johnson et al., 2010).

Paraquat, or 1,1′-dimethyl-4,4′-bipyridilium dichloride, generates ROS in the form of superoxide anions directly through a cyclic redox reaction with oxygen and damages a variety of cellular targets. It is therefore widely used in oxidative stress studies (Bus and Gibson, 1984). Paraquat only releases free oxygen radicals when inside the cell via microsomal NADPH-cytochrome *c* reductase. By inhibiting oxidative phosphorylation pathway complex I (NADH: ubiquinone oxidoreductase) in the mitochondria, paraquat induces oxidative stress, producing superoxide (Fukushima et al., 1994). In addition, paraquat can induce neurotoxicity by oxidative stress and excitotoxicity (Djukic et al., 2012). Oxidative stress induced by paraquat can lead to carbonylation of proteins (Seehuus et al., 2006), DNA damage (Ali et al., 1996), and lipid peroxidation (Suntres, 2002). Paraquat has been originally developed as a contact herbicide because it is a powerful inhibitor of photosynthesis. It is still widely used, although restricted in some countries due to public health concerns. Paraquat generates oxidative stress in a variety of organisms, including honey bees, *Apis mellifera* (Seehuus et al., 2006).

In this study, we characterize the variation in oxidative stress resistance in drone honey bees after paraquat injection by determining the mortality dynamics of treatment and control groups. Additionally, we quantify the levels of oxidative damage to proteins and lipids in different tissues of drones that exhibited low or high resistance to paraquat injection. Resistant drones displayed elevated levels of lipid damage, suggesting that tolerance of molecular damage is primarily responsible for survival of an acute oxidative stress in honey bee drones. This pattern may be compatible with previous studies in honey bee workers and support a similar model of mortality between the drone and worker honey bees, despite their stark differences in life history. Moreover, our results represent another example of a dissociation between functional decline and mortality risk in honey bees (Rueppell et al., 2007) because the individuals with higher levels of oxidative damage survived longer.

2. Materials & methods

2.1. Drone sources

All honey bee (*Apis mellifera*) drones used in this study were reared from colonies near the North Carolina State University Lake Wheeler Honey Bee Research Facility (Raleigh, NC, (GPS coordinates: 35.725°N, 78.676°W)) and the University of North Carolina at Greensboro Honey Bee Research Facility (Greensboro, NC, GPS coordinates: 36.063°N, 79.831°W). In order to maximize the genetic diversity represented in our samples, we included drones from nine different colony sources, including a colony from a breeding program for hygienic behavior (Spivak and Downey, 1998). The majority of drones were from four colonies: the hygienic colony "Dhyg", and colonies "D52", "D57", and "D65". Overall, the individuals used were representative of the commercial honey bee population in the U.S.

2.2. Drone rearing

From each drone source, we collected up to two frames of capped drone brood that were close to adult emergence. Entire drone frames were placed into separate mesh-wire cages that trapped adults as they emerged. These cages were housed in a temperature-controlled incubator set at 33 °C. All adults that emerged during a 2-day period were collected and placed into drone cages (Laidlaw and Page, 1997) that were labeled with a unique source identification code. Each cage was 10 × 10 × 2.5 cm (L × W × H) and consisted of a wood frame, a sheet of metal screen mesh on one side, and a sheet of plastic slotted queen-excluder material on the opposite side. These cages were placed inside of full-sized queen-right host colonies. This setup allowed workers to walk into and out of the cages to care for the adult drones and provided a common maturation environment. All drones were selected randomly and the drone cages were randomly placed into the host colonies. A maximum of 50 drones was placed into each cage and up to nine cages were placed into a single host colony. All collected drones were of approximately the same age (within 2 days) and all were allowed to mature in the host colonies for an additional 10 days before experimental treatment.