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Gene expression differences in relation to age and social environment in queen and worker bumble bees

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ABSTRACT

Eusocial insects provide special insights into the genetic pathways influencing aging because of their long-lived queens and flexible aging schedules. Using qRT-PCR in the primitively eusocial bumble bee *Bombus terrestris* (Linnaeus), we investigated expression levels of four candidate genes associated with taxonomically widespread age-related pathways (*coenzyme Q biosynthesis protein 7*, *COQ7*; *DNA methyltransferase 3*, *Dnmt3*; *foraging*, *for*; and *vitellogenin*, *vg*). In Experiment 1, we tested how expression changes with queen relative age and productivity. We found a significant age-related increase in *COQ7* expression in queen ovary. In brain, all four genes showed higher expression with increasing female (queen plus worker) production, with this relationship strengthening as queen age increased, suggesting a link with the positive association of fecundity and longevity found in eusocial insect queens. In Experiment 2, we tested effects of relative age and social environment (worker removal) in foundress queens and effects of age and reproductive status in workers. In this experiment, workerless queens showed significantly higher *for* expression in brain, as predicted if downregulation of *for* is associated with the cessation of foraging by foundress queens following worker emergence. Workers showed a significant age-related increase in *Dnmt3* expression in fat body, suggesting a novel association between aging and methylation in *B. terrestris*. Ovary activation was associated with significantly higher *vg* expression in fat body and, in younger workers, in brain, consistent with vitellogenin's ancestral role in regulating egg production. Overall, our findings reveal a mixture of novel and conserved features in age-related genetic pathways under primitive eusociality.

1. Introduction

The occurrence of aging in organisms raises important questions at both evolutionary and mechanistic levels (Hughes and Reynolds, 2005; Parker, 2010; Flatt et al., 2013; Gems and Partridge, 2013). Aging is defined as the deterioration in organismal survivorship, fecundity and performance with age. At the mechanistic (proximate) level, much progress have been made in recent years in characterizing the genetic pathways that underpin aging, including those involved in nutrient sensing, energy metabolism, stress and growth (Kenyon, 2010; Gems and Partridge, 2013). The eusocial insects (those with a worker caste), comprising principally the eusocial Hymenoptera and termites, provide a particularly informative case in terms of understanding the genetic pathways and mechanisms that influence aging. First, eusociality is associated with phenotypically flexible aging and longevity. Specifically, the queen and worker castes, which arise from the same genome, exhibit widely differing schedules of aging and longevity, with queens typically far outliving workers (Keller and Genoud, 1997; Keller and Jemielity, 2006; Bourke, 2007; Parker, 2010). Second, eusociality in insects is associated with reversals in conventional life history patterns, as exemplified by positive associations between longevity and lifetime reproductive success observed in queens of eusocial insects (Lopez-Vaamonde et al., 2009; Heinze et al., 2013). In queens of the ant *Cardiocondyla obscurior*, aging-related gene expression changes have been found to occur in a direction opposite to that found in Drosophila, consistent with a reversed (positive) association of longevity and fecundity under eusociality (Von Wyschetzki et al., 2015). Third, in eusocial insects, aging can be regulated by the social environment and even reversed within the lifespan of individuals (Huang and Robinson, 1996; Amdam et al., 2005; Smedal et al., 2009; Amdam, 2011; Woodard et al., 2013). All these traits point, in eusocial insects, to a large degree of flexibility and responsiveness in the genetic pathways that influence aging.

Several major, well-characterized genetic pathways associated with aging in other organisms have been shown to be linked to aging in eusocial Hymenoptera (Parker et al., 2004; Corona et al., 2005, 2007; Amdam, 2011; Von Wyschetzki et al., 2015). For example, in workers

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of the honey bee (*Apis mellifera*), a pathway involving juvenile hormone and vitellogenin has been shown to affect aging and the temporal division of labor (changes in task with time) in workers (Amdam et al., 2004; Nelson et al., 2007; Münch and Amdam, 2010; Bloch and Grozinger, 2011; Page et al., 2012). Since vitellogenin is ancestrally a yolk protein, this suggests that its original role in the regulation of reproduction has been co-opted to social ends during the course of social evolution (Amdam et al., 2004; Remolina and Hughes, 2008; Flatt et al., 2013), but whether a similar process of co-option has occurred in other eusocial Hymenoptera remains unclear (Bloch and Grozinger, 2011; Amsalem et al., 2014; Von Wyschetzki et al., 2015). In *A. mellifera*, DNA methylation has been found to covary with task and age in workers (Herb et al., 2012; Lockett et al., 2012) and with age and caste in larvae (Foret et al., 2012; Shi et al., 2013). Evidence from other eusocial Hymenoptera and termites also suggests a role for DNA methylation in the regulation of caste-specific longevity (Yan et al., 2015). To test whether or not these processes and phenomena are general requires additional studies of genetic mechanisms of aging, including epigenetic effects of the social environment and effects associated with reproduction, in eusocial insects.

We investigated gene expression as a function of age, social environment and reproductive status for a set of candidate genes associated with taxonomically widespread age-related genetic pathways in queens and workers of the bumble bee *Bombus terrestris* (Linnaeus). We followed a tissue-specific approach, quantifying gene expression in brain, fat body or ovary, because previous studies suggest that relevant pathways are localized within these tissues (Grozinger et al., 2007; Thompson et al., 2008; Foret et al., 2009; Page et al., 2012). *B. terrestris* has a more primitive form of eusociality (characterized by lower queen-worker dimorphism in the reproductive system) than the advanced eusocial *A. mellifera* and shares with it a common primitively eusocial ancestor (Cardinal and Danforth, 2011). The potential contrast with *A. mellifera* renders the genetic pathways underpinning aging in *B. terrestris* of particular interest. *B. terrestris* forms annual colonies of a single queen and 100–200 worker daughters. Following eclosion (emergence from the pupa) in the previous year and overwintering diapause, *B. terrestris* queens typically live about 6 months (Goulson, 2010), while workers live 1–2 months as adults in laboratory colonies (Holland and Bourke, 2015). Colonies produce first workers and then (in the reproductive phase) males and new queens. During this second part of the colony cycle, some workers activate their ovaries to become reproductive, egg-laying workers (Duchateau and Velthuis, 1988; Bloch, 1999; Zanette et al., 2012).

We selected four candidate genes, *coenzyme Q biosynthesis protein 7* (*COQ7*), *DNA methyltransferase 3* (*Dnmt3*), *foraging* (*for*) and *vitellogenin* (*vg*), as they combined coverage of a range of putative functions associated with aging with a gene structure suitable for the design of gene expression assays. *COQ7*, also known as *clk-1*, encodes a biosynthesis protein involved in electron transport in the mitochondrial respiration pathway. Mutants for this gene exhibit increased longevity in *Caenorhabditis elegans* (Felkai et al., 1999) and mice (Liu et al., 2005). In *A. mellifera*, *clk-1* expression decreases with age in queens but not workers (Table 1). Mitochondrial respiration is thought to contribute to aging via production of reactive oxygen species (Larsen and Clarke, 2002). However, because there is also evidence against a direct role for oxidative damage in aging (Van Remmen et al., 2003; Parker et al., 2004), we sought to test whether the expression of a gene in the mitochondrial respiration pathway is associated with age in *B. terrestris*.

Dnmt3 encodes the DNA methyltransferase enzyme essential for creating de novo DNA methylation marks on the genome. DNA methylation is known to change with age in mammals (Wilson et al., 1987; Issa, 2003) including humans (Horvath, 2013). In *A. mellifera*, associations of DNA methylation patterns with age in workers (Lockett et al., 2012) show that a link between DNA methylation and aging also occurs in eusocial Hymenoptera. In *B. terrestris*, recent evidence points to an association between DNA methylation and worker reproduction (Amarasinghe et al., 2014). In *Apis cerana*, *Dnmt3* expression changes with age (in workers) and caste (Table 1) and, in *A. mellifera*, there is experimental evidence for its role in downregulating queen development (Kucharski et al., 2008). However, *Dnmt3* expression has not previously been investigated in *B. terrestris*.

In Drosophila, the *for* gene encodes a cGMP-dependent protein kinase and underpins a polymorphism in foraging behavior (Osborne et al., 1997). Foraging kinase also influences whether energy is stored as lipids or carbohydrates and interacts with the insulin pathway (Kent et al., 2009). Consistent with its association with foraging behavior in Drosophila, *for* has been found to be overexpressed in foraging workers compared to nurse workers in several species of eusocial Hymenoptera, including *A. mellifera* and *B. terrestris*, although the pattern is not universal (Table 1). In addition, *for* expression has been found to decrease with age in *B. terrestris* queens and workers (Table 1). In queens, this occurred only in individuals from which workers were removed, implying the presence of an interaction between age and social environment (Woodard et al., 2013). Since foundress *B. terrestris* queens forage externally only up to the time when their first workers eclose (Goulson, 2010), we predicted that *for* expression would be higher in foundress queens experimentally deprived of workers.

The *vg* gene encodes an insect version of a yolk protein. In *C. elegans*, *vg* expression provides a potential example of hyperfunction (Blagosklonny, 2012), whereby *vg* is not downregulated after reproduction as expected, but maintains relatively high expression levels into later life, resulting in detrimental effects (DePina et al., 2011; Gems and Partridge, 2013). In eusocial Hymenoptera, *vg* is known to have developed novel functions, particularly with respect to regulation of temporal division of labor in workers, but when in social evolution such functions arose is unclear (Amdam et al., 2004; Corona et al., 2007; Nelson et al., 2007; Münch and Amdam, 2010; Bloch and Grozinger, 2011; Wurm et al., 2011; Page et al., 2012). *vg* may act by regulating microRNAs (Nunes et al., 2013). *vg* expression is also associated with age and reproductive status in eusocial Hymenoptera (Table 1). In *B. terrestris*, *vg* was recently found to be associated with worker aggression independently of worker ovarian activation (Amsalem et al., 2014), but relationships with queen and worker age remain unclear.

We performed two experiments. In Experiment 1, to test effects of queen age, queens of different relative ages were removed sequentially from colonies and gene expression in brain and ovary was assayed using quantitative real-time PCR (qRT-PCR). Demographic data were also collected from these colonies to investigate associations of gene expression with queen productivity. In Experiment 2, to test effects of queen age, worker age, worker reproductive status and the social environment, queens were reared in either a 'social' treatment (allowed to head colonies) or an 'asocial' treatment (deprived of workers). Gene expression in brain was assayed for queens removed at different relative ages using qRT-PCR. In the social treatment colonies in Experiment 2, marked workers were also sampled at different ages and gene expression in brain and fat body was assayed using qRT-PCR.

2. Materials and methods

2.1. Experimental procedures

2.1.1. Queen gene expression as a function of relative age

Queen gene expression as a function of relative age was investigated in both Experiments 1 and 2. In Experiment 1, queens were sampled only over the reproductive phase of the colony cycle and in Experiment 2 they were sampled over the colony cycle as a whole.

For Experiment 1, we obtained 58 colonies (each containing a single queen with workers and brood) of *Bombus terrestris terrestris* from a commercial supplier (Syngenta Bioline Bees B.V., Weert, The Netherlands) in two cohorts (Cohort 1: 48 colonies obtained on 22 January 2010, mean ± s.d. number of workers = 24 ± 5; Cohort 2: 10 colonies obtained on 11 March 2010, mean ± s.d. number of workers =