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Elevated expression of ageing and immunity genes in queens of the black garden ant

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ABSTRACT

Studies in model organisms have identified a variety of genes whose expression can be experimentally modulated to produce changes in longevity, but whether these genes are the same as those involved in natural variation in lifespan remains unclear. Social insects boast some of the largest lifespan differences known between plastic phenotypes, with queen and worker lifespans differing by an order of magnitude despite no systematic nucleotide sequence differences between them. The contrasting lifespans of queens and workers are thus the result of differences in gene expression. We used RNA sequencing of brains and legs in 1-day-old and 2-month-old individuals of the ant *Lasius niger* to determine whether genes with queen-biased expression are enriched for genes linked to ageing in model organisms. Because the great longevity of queens may require investment into immune processes, we also investigated whether queen-biased genes are enriched for genes with known roles in immunity. Queen-biased genes in legs were enriched for ageing genes and for genes associated with increasing rather than decreasing lifespan. Queen-biased genes in legs were also enriched for immune genes, but only in 1-day-old individuals, perhaps linked to the changing roles of workers with age. Intriguingly, the single most differentially expressed gene between 1-day-old queen and worker brains was an extra-cellular form of CuZn Superoxide Dismutase (SOD3), raising the possibility of an important role of anti-oxidant genes in modulating lifespan.

1. Introduction

The expected lifespan of an organism in the absence of extrinsic mortality is not rigidly determined by its genome, but instead can vary plastically within a species (Fielenbach and Antebi, 2008; Lucas and Keller, 2017). In some taxa, naturally-occurring environmentally-determined polymorphisms can be associated with large differences in lifespan (Flatt et al., 2013), yet the genes involved in this natural plastic variation in many cases remain unknown. Studies in model organisms have revealed many genes whose expression can be experimentally modulated to affect lifespan in the laboratory (Tacutu et al., 2013), but it remains unknown whether these genes are also involved in plastic longevity differences in the wild.

A striking example of natural plastic polymorphisms in longevity is found in advanced social insects such as ants, where females can develop into either queens, which are typically very long-lived (Keller and Genoud, 1997), or workers, which have a shorter lifespan. In most species, queens and workers share a common genome and their different lifespans are therefore regulated through differential gene expression (Schwander et al., 2010). The origins of these expression differences can be due to a range of factors, including epigenetic variation and physiological reactions to different environments. The naturally-occurring polymorphisms in social insects have thus been the focus of study to understand the basis of these plastic differences in longevity and the changes that accompany ageing (Aurori et al., 2014; Jemielity et al., 2007; Lucas and Keller, 2017; Lucas and Keller, 2014; de Verges and Nehring, 2016). Gene expression studies have for the most part focused on a few candidate pathways (Aamodt, 2009; Corona et al., 2005; Corona et al., 2007; Lucas et al., 2016; Parker et al., 2004a) or used transcriptome-wide analysis (Seehuus et al., 2013) but have not systematically investigated the extent of overlap with genes involved in ageing in model organisms.

In this study, we first test the hypothesis that genes related to ageing in model organisms (list obtained from the *GenAge* database, Tacutu et al., 2013) also underlie the large-scale natural plasticity in longevity in the black garden ant *Lasius niger*. Queens of *L. niger* are substantially larger than workers, physiologically specialised for egg-laying, and live up to 30 years (Hölldobler and Wilson, 1990), as compared to only

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3 years for workers (Kramer et al., 2016). After emergence as adults, queens spend a few weeks accumulating nutritional resources before engaging in a mating flight, after which successfully-mated queens found an incipient colony, using their nutritional reserves to feed their first cohort of workers (Hölldobler and Wilson, 1990). Once the first workers are produced, queens stop feeding the brood and become dedicated egg-layers. Workers conduct all the other colony tasks.

Longevity can be affected by investment in the immune system, with higher investment contributing to reduced risk of infection, but carrying costs in the form of energetic demands (Moret and Schmid-Hempel, 2000). The benefit of down-regulating the immune system may therefore depend on the resources available and the environmental hostility (Schmid-Hempel, 2005). In laboratory-reared *Drosophila*, down-regulation of immune gene expression was found in lines with extended longevity (Carnes et al., 2015), but this may be due to the lack of immune threats in a laboratory setting. Whether the long life of social insect queens is linked to changes in immunity gene expression remains unknown. In honeybees, results suggest that worker pupae have higher levels of Prophenoloxidase (PPO) expression than queen pupae (Lourenço et al., 2005) and adult queens may have higher Phenoloxidase (PO) activity than workers (Schmid et al., 2008). PPO is the molecular precursor to PO, which regulates the melanisation response, an important aspect of the insect immune system (González-Santoyo and Córdoba-Aguilar, 2012). However, insect immunity is a multi-faceted system involving several pathways of humoral and cellular responses (Lemaitre and Hoffmann, 2007). Instead of focusing on a single pathway, we therefore investigate whether queens show increased expression of genes linked to immunity by globally studying the expression of genes obtained from an extensive database of insect immunity genes (Brucker et al., 2012).

These targeted analyses of genes linked to ageing and immunity investigate whether the longevity of queens is associated with a concerted shift in the expression of a large group of genes. Because individual genes may also play crucial roles in queen longevity, we also identified the genes that show the strongest patterns of differential expression between queens and workers.

To perform these analyses of gene expression, we chose two tissues. First, we chose the brain because it affects many traits linked to survival and organismal function. Second, we chose legs as they mostly comprise muscles that perform similar tasks in queens and workers, and should thus allow us to identify genes whose expression is inherently different between the two castes, rather than genes associated with these different roles. We measured gene expression in 1-day-old and 2-month-old individuals to represent two divergent points in development. One-day-old queens and workers have recently emerged from the pupal stage and are thus very young adults. By contrast, 2-month-old workers are fully developed and conduct typical worker tasks. Queens of this age are also in a “typical” physiological state where they have initiated egg production. We did not use older individuals in order to avoid confounding the differences between castes with those caused by differential rates of ageing in queens and workers. Using an age at which workers could be considered to be old while queens are still young would make it impossible to differentiate the causes of differential ageing from its consequences.

2. Results

Overall, substantially fewer genes were significantly differentially-expressed between queens and worker in brains than in legs. Out of the 63,661 transcriptome components, 1384 (2.2%) were differentially expressed in 1-day-old brains and 486 (0.8%) were differentially expressed in 2-month-old brains. By contrast, these values were 5792 (9.1%) in 1-day-old legs and 10,400 (16.3%) in 2-month-old legs (Table 1). Furthermore, differentially-expressed genes in brains were more frequently queen-biased than worker-biased (binomial tests, 1-day-old: $P < 0.0001$, 2-month-old: $P < 0.0001$), while in legs they were more frequently worker-biased than queen-biased (binomial test, 1-day-old: $P < 0.0001$, 2-month-old: $P < 0.0001$; Table 1).

2.1. Differential expression of ageing genes

A total of 429 homologs of the ageing genes listed in the *GenAge* database (Tacutu et al., 2013) were identified in *L. niger* by strict reciprocal blast. In each of the four age/tissue combinations (1-day-old legs, 1-day-old brains, 2-month-old legs, 2-month old brains), there were proportionally more ageing genes among queen-biased genes than among worker-biased genes (Table 1, Supplementary Fig. S1), but the difference was significant only in the legs of 1-day-old individuals (Fisher's exact test; 1-day-old legs: $P < 0.0001$; 2-month-old legs: $P = 0.58$, 1-day-old brains: $P = 0.47$, 2-month-old brains: $P = 0.58$).

Out of the 2651 genes that were queen-biased in 1-day-old legs, 96 (3.6%) were ageing genes, as compared to 17 out of 1009 (1.7%) in 1-day-old brains, 71 out of 4261 (1.7%) in 2-month-old legs and four out of 356 (1.1%) in 2-month-old brains. These differences were significant between 1-day-old legs and each of the other three categories (Fisher's exact test vs. 1-day-old brains: $P = 0.0018$, vs. 2-month-old legs: $P < 0.0001$, vs. 2-month-old brains: $P = 0.011$). Out of 6139 genes that were worker-biased in 2-month-old legs, 93 (1.5%) were ageing genes, as compared to four out of 375 (1.1%) in 1-day-old brains, 34 out of 3141 (1.1%) in 1-day-old legs and 0 out of 130 (0%) in 2-month-old brains. None of these differences were significant.

The *GenAge* database provides information on whether increasing a gene's expression is associated with increased lifespan (“pro-longevity”) or decreased lifespan (“anti-longevity”). In legs, the ratio of pro-longevity to anti-longevity genes was higher in queen-biased genes than in worker-biased genes both in 1-day-old and 2-month-old individuals (Table 2), although the difference was only significant in 2-month-old individuals (Fisher's exact test, 1-day-old: $P = 0.4$, 2-month-old: $P = 0.036$). In brains, there was little statistical power because there were only very few worker-biased genes (Table 2).

Using a less strict reciprocal blast, homologs of a further 308 genes from the *GenAge* database were identified in *L. niger*, leading to a total of 737 genes. Of these, 24 were consistently worker-biased and 25 were consistently queen-biased within an age or tissue (Supplementary Data S2), compared to 1145 and 475 non-ageing genes that were consistently worker- and queen-biased respectively. The consistently queen-biased ageing genes include two anti-oxidant genes that showed consistent queen-biased expression (Peroxiredoxin 1/Thioredoxin Peroxidase 1 was queen-biased in both legs and brains of 1-day-old individuals; Glutathione Peroxidase 2 was queen-biased in both legs and brains of 2-month-old individuals, Supplementary Data S2).

2.2. Differential expression of immunity genes

A total of 86 homologs of the immunity genes listed in the Insect Innate Immunity Database (IIID) (Brucker et al., 2012), were identified in *L. niger* by strict reciprocal blast. In 1-day-old legs, 1-day-old brains and 2-month-old legs, there were proportionally more immunity genes among queen-biased genes than among worker-biased genes (Table 1, Supplementary Fig. S2), the difference being significant in 1-day-old legs (Fisher's exact test; 1-day-old legs: $P = 0.0007$; 1-day-old brains: $P = 0.2$; 2-month-old legs: $P = 0.31$). In 2-month-old brains, there was no difference in the proportion of immunity genes between queen-biased and worker-biased genes (Fisher's exact test; $P = 1$).

Out of the 356 genes that were queen-biased in 2-month-old brains, five (1.4%) were immunity genes, as compared to 11 out of 1009 (1.1%) in 1-day-old brains, 23 out of 2651 (0.9%) in 1-day-old legs and 18 out of 4261 (0.4%) in 2-month-old legs. These differences were only significant between 2-month-old legs and the other three categories (Fisher's exact test vs. 2-month-old brains: $P = 0.028$, vs. 1-day-old legs: $P = 0.024$, vs. 1-day-old brains: $P = 0.016$). Out of the 130 worker-biased genes in 2-month-old brains, two (1.5%) were immunity genes,